REMARKS

The foregoing revisions adds, changes, or deletes claims in this application. A detailed listing is provided, with appropriate status identifiers, of all claims that are or were pending. Upon entry of the amendments, claims 1 and 14-19 will be pending. Applicants respectfully request entry of the foregoing amendments and reconsideration of the application in view of the following remarks.

Summary of Amendments

The title of the invention has been amended to more closely reflect the elected subject matter, as requested by the Examiner.

Paragraph [0033] of the specification has been amended to update the reference to the patent application with the corresponding patent number, as requested by the Examiner, and to correct a clerical error.

Claim 1 has been amended to recite only the elected invention. Claims 2, 3, 11 and 12 are canceled without prejudice or disclaimer as being redundant in view of the elected invention recited in amended claim 1.

Claims 4-10 and 13 are canceled without prejudice or disclaimer as being drawn to non-elected subject matter. Applicants reserve the right to file one or more divisional applications to the subject matters of these claims with the same rights of priority as the instant application.

Claim 14 has been amended with regard to the recited types of immune compromised humans.

Response to Objections

The Office Action objects to dependent claims 2, 3, 11, and 12 for failing to limit the base claim in view of the response to the restriction requirement. The cancellation of these claims obviates this objection.

The Office Action objects to claims 1, 11, 12 and 14-19 as directed to non-elected subject matter. As noted, claims 11 and 12 are canceled. Claim 1 is amended to recite the elected invention. Applicants believe that this amendment obviates the objection to claims 1 and 14-19.

Response to Indefiniteness Rejection

Claims 2 and 3 were rejected for alleged indefiniteness. The cancellation of these claims obviates that rejection.

Response to Non-enablement Rejection

The Office Action rejects the claims under the first paragraph of 35 USC § 112, alleging the specification is non-enabling with respect to the recitation of an "immune compromised human." Applicants respectfully traverse this rejection.

Although the Office Action agrees that the specification *is* enabling as to immune-compromised humans with end-stage renal disease (ESRD), it questions enablement as to other immune compromised humans, such as those recited in claim 14. Thus, the Office Action asserts at page 6 that the "specification fails to teach that other severely immunocompromised patients such as the claimed cancer patients, AIDS patients, diabetic patients, elderly, patients or immunosuppressive therapy, transplant patients, patients with surgical procedures, burn patients, and other patients in acute care settings have the ability to elicit IgG that binds Type 5 and/or Type 8 capsular polysaccharide of *S. aureus* in an amount sufficient to provide for protection from infection/disease."

Yet, in paragraph [0035] at pages 11 and 12, the specification does teach that these types of immune compromised humans will "benefit from the administration of vaccines according the present invention." Those skilled in the art would have understood that, in order to be beneficial, the vaccine would have to elicit a protective immune response. In effect, therefore, the specification provides the very teaching that the Office Action asserts is missing.

The Office Action further contends that "[t]here is no demonstration of protective immunity or elicitation of antibodies upon administration of the claimed conjugate in humans or in any animal model correlative of the claimed immune-compromised patients." Again, the Office Action overlooks disclosures in the specification that are significant in this regard.

For instance, the specification includes detailed examples of the administration of a vaccine of the present invention to ESRD patients. Paragraph 19 at page 7 teaches why ESRD patients are representative of immune-compromised patients. For instance, many ESRD patients are unable to fix complement and may have phagocytes with weakened chemotactic movement. ESRD patients on hemodialysis often suffer from uremia, which further impacts the functionality of granulocytes and

complement fixation. Diabetes and uremia also impact the functionality of B cells. In essence, Applicants chose ESRD patients to study the efficacy of the invention because ESRD patients represent a "worst-case scenario" of immune-compromised patients. With the showing that the invention works in ESRD patients, no undue amount of experimentation would be required to practice the invention with respect to other immune-compromised patients.

Read against the backdrop of the whole specification, the examples with ESRD patients provide ample guidance for the skilled person to make and use a vaccine of the present invention to induce a qualitatively similar immune response in ***any*** immune compromised humans, regardless of the etiology of immune compromised condition. Accordingly, beyond enabling methodology for protecting ESRD patients from infection, as the Examiner acknowledges, the specification also provides support for protecting other immune compromised humans.

Applicants note further that the ESRD patients involved in the trials reported in the specification included diabetic patients (52% of patients) and elderly patients (mean age = 58.3 years). *See, e.g.,* paragraph 53 at page 16 of the specification. Additionally, as reported by Shinefield *et al.,* in a *New England Journal of Medicine* article on this trial (Document A5 of the IDS submitted August 12, 2003), 69% of patients had vascular access through a graft, *i.e.*, an implanted device. Thus, data from this trial underscore the enabling quality of the present specification vis-à-vis claim scope related to diabetic patients, elderly patients, and patients with invasive surgical procedures, as well as to transplant patients (many ESRD patients also have received transplants) and patients in acute-care settings.

In view of Applicants' objective teachings that their claimed invention is useful for immune compromised humans generally, plus the examples relating to ESRD patients specifically, the Examiner cannot make out a proper rejection for lack of enablement without providing "evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility." *In re Cortright,* 165 F.3d 1363 (Fed. Cir. 1999). The only basis cited in the Office Action is the teaching, drawn from an earlier publication by the inventors, stating that "active immunization is unlikely to work in low birth weight neonates, or cancer, AIDS and burn patients who are strongly immunocompromised."

This sentence is quoted out of context, however. The next sentence states that "[h]yperimmune immunoglobulin preparations would probably be the treatment of choice for preventing or treating *S. aureus* infections in such high-risk, immunocompromised individuals."

Thus, the publication does not suggest that either neonates or patients suffering cancer, AIDS or burn injury are beyond protection by immunological means. The cited passage is speculative in any event and, hence, would not have impressed those skilled in the art as an authoritative statement on the types of immune compromised humans for which an *S. aureus* vaccine would be useful. Finally, the cited passage does not address the ability of the specific vaccine of the present invention, which comprises glycoconjugates of both Type 5 and Type 8 polysaccharide antigens of *S. aureus*, to provide protection. Thus, those skilled in the art would not have read the publication to cast doubt on the express teachings of the present application regarding the usefulness of the claimed invention in protecting immune compromised humans against infection.

In order to expedite prosecution, Applicants have amended claim 14 to cancel the specific recitation of neonates, AIDS patients, and burn patients. With regard to cancer patients, Applicants submit herewith a paper reporting the successful vaccinations of cancer patients, Chisolm *et al.*, *Arch. Dis. Child* 84: 496-500 (2001). Chisolm reports that 66% of cancer patients immunized against influenza made some protective response to the vaccine and 55% showed protective antibodies to all three viral strains of the vaccine. In the Abstract, the authors state that the data "support[] annual vaccination for children being treated for cancer." This paper provides hard data contradicting the speculation in the cited reference, and demonstrates that active immunization can be effective in cancer patients. In view of this evidence, there is no objective reason to doubt the asserted utility of the claimed invention in non-ESRD patients, and the enablement rejection should be withdrawn.

Response to Prior Art Rejections

The Office Action rejects the claims under 35 USC §102 and 35 USC § 103, citing several different references. Applicants respectfully traverses these rejections.

At the outset, Applicants wish to emphasize that none of the cited references teach a method of ***protecting*** an immune-compromised human from *Staphylococcal* and/or *Enterococcal* bacterial infection. Although some references discloses compositions that are reported to induce antibodies against *Staphylococcal* and/or *Enterococcal* antigens, there is no reasonable basis for assuming that those compositions are ***protective***. Although the identification of compositions that can induce antibodies is an important step in vaccine development, it is not the final step.

The relationship between immunogenicity and protection always is uncertain, and that uncertainty is especially pronounced where (a) the population to be vaccinated is immune

compromised and/or (b) the vaccine is based on a polysaccharide antigen. The fact that protection of immune-compromised individuals via vaccination is an uncertain proposition at best is highlighted in Fattom *et al.*, *Annals of Med.* (1996), which notes at page 45 the "conflicting data from animal models regarding the protection value of antibodies to *S. aureus*" and which concludes at page 46 by cautioning that "these impressive and long-awaited results in animals may or may not be predictive of the value of active and passive immunization in humans." (A list is appended of additional articles on this subject.) The situation is exacerbated for vaccines based on polysaccharide antigens, which are widely-known for their poor immunogenicity (specification at page 3, last paragraph); that is, polysaccharide antigen-based vaccines effective in normal populations often fail in immune-compromised populations.

Applicants are submitting herewith two additional papers which evidence the significant difference between the production of antibodies and protection against infection, Romero-Steiner *et al.*, *Clin. Infect. Dis.* 29: 281-88 (1999), and Musher *et al.*, *J. Infect. Dis.* 182: 158-67 (2000).

Romero-Steiner analyzed antibodies produced in response to an anti-*Streptococcal* polysaccharide vaccine, and found that the IgG produced in healthy elderly patients had significantly reduced opsonophagocytic activity than IgG produced in younger adults. Thus, even though the elderly patients produced anti-pneumococcal antibodies in response to the vaccine, the antibodies may not have been sufficiently functional to confer protection against infection.

Musher reports the results of a study that evaluated antibodies to capsular polysaccharide (CPS) of *Streptococcus pneumoniae* in patients being admitted to a hospital. As summarized in the Abstract, Musher found that the patients had IgG to CPS but that the IgG had a diminished capacity to opsonize the infecting serotype for phagocytosis, as compared to anti-CPS IgG from a control population. Thus, while the patients had antibodies to CPS, the antibodies were poorly functional and not protecting them from infection.

According to Musher, the data explain "why antibody level can not serve as a surrogate for immunity: the degree of immunity may vary greatly, depending on the functional activity of the antibody." Page 165, col. 1. Musher also states that the data "underscore the importance of using functional assays, rather than simply measuring IgG levels by ELISA, for studying and comparing polysaccharide vaccines." *Id.* (last sentence).

Musher and Romero-Steiner demonstrate that a composition that is able to induce antibodies to a given antigen is not necessarily going to confer protection against infection by the antigen. In view of this evidence, the position expressed in the Office Action, that a vaccine that has been shown to induce antibodies but has not been shown to be protective nevertheless anticipates a protective vaccine, is unsupportable. Because it is only Applicants who have disclosed a method of ***protecting*** an immune-compromised human from *Staphylococcal* and/or *Enterococcal* bacterial infection, the instant prior-art rejections should be reconsidered and withdrawn.

The specific rejections set forth in the Action are addressed in turn below.

1. § 102 Rejection of Claim 2 over Welch *et al.*

Claim 2 is rejected over Welch *et al.* As noted above, claim 2 is canceled for redundancy over amended claim 1. Welch does not suggest the invention of claim 1, because the publication does not implicate protecting an immune compromised human from bacterial infection using a vaccine comprised of glycoconjugates of both Type 5 and Type 8 polysaccharide antigens of *S. aureus*, as recited in claim 1.

2. § 102 Rejection of Claims 1, 2, 3, 11, 14, 15 and 17 over Fattom *et al.*

Claims 1, 2, 3, 11, 14, 15, and 17 stand rejected over Fattom *et al.* (1996), *supra*. The Office Action notes that Fattom refers to a clinical trial of a bivalent vaccine, and concludes that the authors "teach the administration of the instantly claimed bivalent type 5 and type 8 conjugate vaccine." Yet, the Fattom publication does not identify the components of the "bivalent *S. aureus* vaccine" in question, let alone teach that the vaccine comprises glycoconjugates of both Type 5 and Type 8 polysaccharide antigens of *S. aureus*, as recited in claim 1. Thus, this reference simply does not disclose the claimed invention, and this rejection should be withdrawn.

Additionally, this reference fails to teach a method of protecting immune-compromised humans from infection. The reference states merely that a clinical trial "is under way." In fact, that clinical trial (designated UNX-1353) failed to show protection. The trial was a Phase 2 clinical trial conducted by Applicants to assess the efficacy of a formulation containing type 5 and type 8 antigens, each of which were conjugated to *r*EPA. A randomized, double-blind, placebo-controlled design was utilized, and the trial was conducted at 23 academic or academic-affiliated dialysis centers throughout the U.S. Adult patients were stratified by the presence or absence of *S. aureus* nasal carriage. The primary endpoints were the frequencies of *S. aureus* peritonitis and all *S. aureus*

dialysis-related infections (peritonitis plus catheter and/or catheter tunnel infections) in one year of follow-up after immunization.

The trial was initiated in August of 1993. A total of 237 subjects were randomized to the vaccine or placebo treatment in a ratio of 1:1. One hundred twenty (120) received 23 μg of type 5 conjugate and 13 μg of type 8 conjugate in a single 0.5 mL IM dose of vaccine; 117 received an equivalent volume of saline placebo. Two hundred five (205) subjects completed the trial. Twenty-three (23) were lost to death and nine were lost to follow-up for other reasons (these losses were approximately equally distributed between the treatment groups). All 237 subjects were included in the analysis.

There were no significant differences between the treatment groups in age, gender distribution, baseline Karnofsky score, baseline rate of *S. aureus* nasal carriage, or prevalence of diabetes mellitus. There was no evidence of vaccine-related safety concerns.

The primary efficacy analysis revealed no effect on the frequencies of *S. aureus* peritonitis or all *S. aureus* dialysis-related infections. Other infections due to *S. aureus* were too infrequent to permit meaningful analysis. Thus, the clinical trial mentioned in Fattom *et al.* did not demonstrate that the vaccine could protect immune-compromised individuals from infection.[1]

3. § 102 Rejection of Claims 1, 2, 3, 11, 14, 15 and 17 over *Vaccine Weekly*

Claims 1, 2, 3, 11, 14, 15, and 17 are rejected over the *Vaccine Weekly* publication, which is a press release from the assignee of the instant application. The item was cited for reporting "positive results of its ongoing Phase II Dosing Study of StaphVAX (S. aureus type 5 and 8 Capsular Polysaccharide Conjugate Vaccine)." This item fails to anticipate the claimed invention for several reasons.

First, according to the publication, the vaccine is "propriety," and there is no description of its particular components. Second, in keeping with its notation that that the vaccine is "proprietary," it is apparent that the publication purposefully omits a full description of the StaphVAX vaccine and, therefore, does not enable the claimed invention, as required for a reference cited in a § 102 rejection. For example, the item does not describe the vaccine components, how it was made, or how it was

[1] Despite this failure, Applicants pressed on. Although there was nothing in the art to suggest that a higher vaccine does would resolve the lack of efficacy, Applicants planned another trial using higher vaccine doses than were used in UNX-1353. That trial was successful, as reported in the examples of the instant specification.

used (*i.e.*, the dosages or routes of administration tested). Thus, the publication does not place the claimed method in the public domain. For this reason, the rejection is improper and should be withdrawn.

Additionally, the item does not give any indication that the vaccine was protective. The reported Phase II Dosing study related to safety, not efficacy as a protective vaccine. Although the item reports that the vaccine "demonstrated the ability to stimulate significant levels of staph fighting antibodies," this statement does not suggest that the vaccine is protective. As discussed above, the ability to stimulate antibodies does not necessarily correlate with protection. Because the item does not disclose a method of protecting immune-compromised patients against infection, this rejection should be withdrawn.

4. § 103 Rejection of Claims 1, 2, 3, 11, 14 and 15-19 over Fattom *et al.* and Grabstein

Claims 1, 2, 3, 11, and 14-19 are rejected for obviousness over Fattom *et al.* (Annals of Med., 1996) and Grabstein. In making this rejection, the Office Action confuses teachings in Fattom regarding vaccines comprising either Type 5 antigen or Type 8 antigen and the reference in Fattom to a bivalent vaccine. For example, the statements in Fattom regarding the efficacy of "the conjugate vaccine" cited at Page 10 of the Office Action refer to the efficacy of an "*S. aureus* monovalent type 5-rEPA conjugate," not to a vaccine comprising glycoconjugates of both Type 5 and Type 8 polysaccharide antigens of *S. aureus*, as presently claimed.

As noted above, the only "teaching" in Fattom related to a bivalent vaccine is a reference to "[a] clinical trial to evaluate the immune response and efficacy of a bivalent *S. aureus* vaccine." There is no description of the vaccine components, and no teaching that supports the Examiner's assumption that the bivalent vaccine is the same as the vaccine recited in the instant claims, or that it is protective. Thus, it is factually incorrect to assert that Fattom differs from the instant claims only in that Fattom does not teach administration to hemodialysis patients and that Fattom does not include adjuvants or immune stimulants. To the contrary, Fattom differs significantly from the present claims because it simply does not teach the instantly recited method.

Combining Fattom with Grabstein does not render the claimed invention obvious. Grabstein is generally directed to the use of IL-15 as a vaccine adjuvant alone or in combination with other cytokine adjuvants. Grabstein does not teach or suggest any method for protecting immune compromised humans from at least one of *Staphylococcal* and *Enterococcal* infection, and does not teach or suggest a vaccine comprising glycoconjugates of both Type 5 and Type 8 polysaccharide

antigens of *S. aureus*, as recited in the instant claims. Thus, this combination of references does not suggest the claimed invention, and the § 103 rejection should be withdrawn.

5. § 103 Rejection of Claims 1, 2, 3, 11, and 14-18 over Fattom *et al.* (Annals of Med.) and Fattom *et al.* (Vaccine)

Claims 1, 2, 3, 11, and 14-18 are rejected under §103 over Fattom *et al.* (Annals of Med, 1996), discussed above, and Fattom *et al.* (Vaccine, 1995), which is cited for teaching that adjuvants such as monophosphoryl lipid A, QS21 and Novasomes™ were able to increase antibody levels. Like Fattom *et al.* (Annals of Med), Fattom *et al.* (Vaccine) fails to teach or suggest a method for ***protecting*** immune compromised humans from at least one of *Staphylococcal* and *Enterococcal* infection. Although the cited combination of references might have suggested a vaccine comprising an adjuvant, it would not have presaged the protective method recited in the instant claims. Accordingly, the § 103 rejection over these references should be withdrawn.

For the forgoing reasons, Applicant believes that the application is in condition for allowance, and an early notice to that effect is requested. Should there be any questions regarding this submission, or should any issues remain, the Examiner is invited to contact the undersigned by telephone.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 CFR §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 CFR §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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Articles relating to protection of immune-compromised individuals

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Response to influenza immunisation during treatment for cancer

J C Chisholm, T Devine, A Charlett, C R Pinkerton, M Zambon

Abstract

***Aims*—To assess the annual risk of influenza infection in children with cancer and the immunogenicity of a trivalent split virus influenza vaccine in these children.**

***Methods*—Eighty four children with cancer were tested for susceptibility to the circulating strains of influenza virus in autumn 1995 and 1996. Non-immunised children were reassessed the following spring for serological evidence of natural infection. Forty two patients received two doses of influenza vaccine. These children were receiving continuing chemotherapy for acute lymphoblastic leukaemia or were within six months of completing chemotherapy.**

***Results*—Among the 84 children tested for influenza virus susceptibility only 8% of patients were fully protected (antibody titres ≥ 40) against all three of the prevalent influenza virus strains; 33% were susceptible to all three viruses. Evidence of acquired natural infection was seen in 30% of unimmunised patients. Among immunised susceptible patients, 66% made some protective response to the vaccine and 55% showed protective antibody titres to all three viral strains following vaccination. Older age was associated with increased response to the H1N1 and H3N2 vaccine components, but total white cell count or neutrophil count at immunisation, type of cancer, or length of time on treatment for acute lymphoblastic leukaemia did not affect response.**

***Conclusions*—Most children with cancer studied were at risk of influenza infection. A significant response to immunisation was seen, supporting annual influenza vaccination for children being treated for cancer.**

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Keywords: influenza; immunisation; susceptibility; cancer

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Influenza is a common cause of respiratory tract infection and hospitalisation during the influenza season in adult patients with leukaemia,[1 2] and following bone marrow transplantation,[3 4] where a high incidence of complications, especially pneumonia, and significant mortality have been reported. Annual influenza infection rates in children vary from 18% to 48%,[5] but the infection is more common in children with cancer than in healthy control children.[6] Although in children with cancer the illness usually runs a mild course it may result in hospitalisation and the interruption of cancer therapy.[6 7] Severe and fatal complications have also been reported in these children.[6–8]

British[9] and American[10] guidelines recommend annual influenza vaccination of high risk groups, including immunosuppressed children and adults, but uptake in high risk patients remains low.[5 11] Contributing to the poor uptake is uncertainty over the efficacy of the vaccine in certain patient groups.[5] Indeed, previous data suggest an impaired response to influenza immunisation in children on treatment for cancer compared with healthy children.[12]

This study is the first to report on the response to a trivalent split virion influenza vaccine in children during cancer therapy. The inclusion of a control, unvaccinated group in whom paired sera were obtained allowed assessment of the annual risk of disease in our population.

Methods

The study was undertaken over two successive influenza seasons, 1995–96 and 1996–97, at the Royal Marsden Hospital. Ethical approval for the study was obtained from the hospital ethics committee and informed, written consent was obtained from each child's parent.

In the first year an unselected group of all consenting patients attending the paediatric oncology outpatient department or resident on the paediatric inpatient ward between 28 September 1995 and 20 October 1995 was tested for susceptibility to the prevalent strains of influenza virus (A/Taiwan/1/86 [H1N1], A/Johannesburg/34/94 [H3N2], and B/Beijing/184/93 [B]). Susceptibility was defined as antibody titre < 40 (see below). Follow up serum was taken in May 1996 to document seroconversion in unimmunised patients. No attempt was made to document respiratory infections in these patients.

Among patients showing susceptibility to one or more of the prevalent virus strains, the following subgroups were offered immunisation in November 1995: (1) patients with acute lymphoblastic leukaemia (ALL) between weeks 9 and 18 of the UKALL XI treatment schedule (that is, on maintenance chemotherapy, between first and second intensification blocks); (2) patients with ALL between weeks 24 and 100 of the UKALL XI treatment schedule except those receiving the third intensification block (that is, patients on continuing maintenance chemotherapy); and (3) all patients who had finished chemotherapy within the past six months (including patients with leukaemia, solid tumours, and bone marrow transplant recipients). The following

children were ineligible for vaccination: age less than 6 months; neutropenic (less than 1.0×10^9/l) or lymphopenic (less than 1.0×10^9/l) at time of vaccination; previously vaccinated against influenza; or egg allergy. Serum was taken to assess response four to six weeks after the second vaccination.

In the second study year vaccination was offered to the same subgroups of patients as above. The vaccine was also given to one patient with relapsed ALL and one child receiving chemotherapy for a brain tumour. Previously vaccinated patients were excluded. Prevaccination and 4–6 week post-vaccination sera were obtained, although, in this cohort, the vaccine was given without knowing the immune status.

All immunised children received two doses of inactivated influenza vaccine (split virion; Aventis Pasteur MSD), subcutaneously, four weeks apart (0.5 ml for children over 4 years and 0.25 ml for children ≤ 4 years). In 1995 the influenza vaccine strains were A/Taiwan/1/86, A/Johannesburg/34/94, and B/Beijing/184/93 according to the recommendations of the World Health Organisation. In 1996, A/Wuhan/359/95 replaced the previous H3N2 component.

Sera were analysed in the Enteric, Respiratory, and Neurological Virus Laboratory, Central Public Health Laboratory, Colindale. Antibodies to the prevalent A and B strains were tested by haemagglutination inhibition (HI). Responses were assessed in terms of geometric mean titre (GMT) and protective response. Antibody levels were expressed on a doubling scale (10, 20, 40, 80, etc) with a level of 40 or more considered as protective.

STATISTICS

McNemar's test of paired proportions was used to assess increase in proportion of children exhibiting a protective response. For each strain the child was placed into one of three categories: immune prior to vaccination; protective response made to vaccine; and susceptible after vaccination. Associations with the response made to the vaccine were assessed using the Kruskal–Wallis test and Fisher's exact test for continuous and categorical variables respectively.

A within subject linear model was used to assess increase in antibody titre. The dependent variable in this analysis was the HI titre, which was $\log_2$ transformed to remove skewness in this variable. For sera where the antibody level was below the lower limit of detection (HI = 10) a value of 5 was used in this analysis. A blocking factor was fitted for subject. The pre/post-vaccination factor and the interaction between this factor and the variables that may influence response were fitted as within subject effects. The assumption of normally distributed residuals and equality of residual variance were assessed using the Shapiro–Francia W' test and the Cook–Weisburg test respectively.

Table 1 Diagnoses of children in study

Diagnosis	Immunised children	Paired sera, non-immunised	Single serum,* non-immunised
On treatment			
ALL	33	6	1
Relapsed ALL	1	0	0
AML	0	2	3
Solid tumours	1	10	8
Off treatment			
ALL	4	1	1
AML	1	1	1
Solid tumours	2	2	1
BMT recipients	0	5*	0
Total	42	27	15

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BMT, bone marrow transplant.
*All allogeneic BMT for acute or chronic leukaemia.

Results

Sera were taken from 67 children in 1995. Of these children, 25 fulfilled the criteria for vaccination and received influenza vaccine. Paired sera were obtained in 27/42 children who were not eligible for immunisation. The remaining 15 children had no follow up serum taken. Twelve of these children died from progressive disease, one was followed up elsewhere, and in two a follow up sample was never taken. A further 17 children were immunised in 1996. Table 1 shows diagnoses of children in the study.

SUSCEPTIBILITY ANALYSIS

Among 84 children tested, 60 (71%) were susceptible to H1N1, 47 (56%) to H3N2, and 58 (69%) to B. Although the greatest pre-existing protection was to H3N2, half of all patients tested were susceptible to this virus. Twenty eight patients (33%) were susceptible to all three viruses and only seven patients (8%) showed protective titres against all three viruses prior to immunisation (data not shown).

Among the 27 unimmunised patients in whom paired sera were available, eight of 26 (30%) patients susceptible to at least one virus showed evidence of exposure to influenza A over the winter months (four to H1N1, four to H3N2), developing antibody where none was detected previously. Only one of these patients had a blood product transfusion in the three months before the second serum sample, indicating that seroconversion was the result of natural infection.

Ten of 19 (53%) unimmunised patients with pre-existing full or partial protection lost protective antibody levels to one or more virus types over the winter months. Five patients had blood or platelet transfusions in the two weeks before the initial blood test and may have had false positive tests on the initial screening. However, the other five patients had no history of recent blood product transfusion.

RESPONSE TO IMMUNISATION

Forty two patients were immunised with influenza vaccine. No adverse events were reported. Prior to vaccination, only four patients (5%) were fully protected against all three viruses (all had been immunised in 1996 without knowing status), whereas post-vaccination 23 patients (55%) were fully protected. Partial protective responses to immunisation were seen in a

Table 2 Numbers of children making response to each virus subunit

		Pre-vaccination	Post-vaccination	
		HI < 40	*HI ≥ 40*	*Total*
H1N1	HI < 40	15	14	29
	HI ≥ 40	0	13	13
	Total	15	27	42
H3N2	HI < 40	7	16	23
	HI ≥ 40	0	19	19
	Total	7	35	42
B	HI < 40	10	18	28
	HI ≥ 40	0	14	14
	Total	10	32	42

Table 3 Response to immunisation by geometric mean titres

	H1N1	*H3N2*	*B*
Pre-vaccination			
Geometric mean	12.6	23.2	12.0
95% CI	8.6 to 19.2	13.2 to 40.8	8.6 to 16.7
Post-vaccination			
Geometric mean	60.4	124.9	48.0
95% CI	32.4 to 112.8	72.0 to 216.0	30.0 to 76.7
p value	<0.0001	<0.0001	<0.0001

further six patients (data not shown). Thus 25/38 patients (66%) susceptible to at least one virus made some protective response to the vaccine.

Table 2 shows the numbers of children responding to each virus subunit. The estimated increases in percentage protected against each virus type post-vaccination were: 33% for H1N1 (95% confidence interval (CI) 17% to 50%, p = 0.0002); 38% for H3N2 (95% CI 21% to 55%, p = 0.0001); 43% for B (95% CI 26% to 60%, p < 0.0001). Analysis of changes in GMT to each virus following immunisation confirmed a significant response to each virus subunit (table 3).

The data were analysed further for factors that might affect response to immunisation. The median age at vaccination of responders was significantly higher than that of non-responders for H1N1 and H3N2 (7 years *v* 3 years, p = 0.03 for both groups) but there was no effect of age on response to B (median age 3 years for responders and non-responders, p = 0.4). There was no correlation between age and the number of protective responses (0, 1, 2, or 3) made to the vaccine (p = 0.9). Whereas mean HI titres prevaccination were independent of age, older children showed higher mean post-vaccination titres to H1N1 and H3N2 but not to B (not shown).

Median total white blood cell count and neutrophil count at the time of immunisation were not significantly different in non-responders and responders to each virus strain and did not correlate with the number of protective responses obtained (data not shown). However, for the H3N2 strain only children with solid tumours produced a higher post-vaccination mean HI titre than those with leukaemia (40.3 *v* 4.6 fold increase), and those off treatment produced a better response than those on treatment (19.5 *v* 4.2 fold increase). In neither case was the difference significant (p = 0.06), but the numbers of patients in the solid tumour and off-treatment groups were small.

Discussion

This study confirms previous reports that children with cancer are highly susceptible to influenza virus. Our estimated natural infection rate of 30% in unimmunised children is similar to that of Borella and Webster,[15] who reported that 46% of unimmunised children with leukaemia suffered with a flu like illness in one influenza season. The loss of protective antibody levels in some unimmunised patients noted in this study, possibly as the result of immunosuppressive therapy, might contribute to the increased the risk of clinical infection in children with cancer noted in previous studies.[7 13]

Previous studies of influenza vaccination in children with cancer have used a variety of different vaccines and have involved both children on treatment for cancer and children who have completed their treatment.[13–22] All these studies have shown the safety of the vaccine in this patient group. However, the immunogenicity of influenza vaccine in children on chemotherapy varies in different reports according to the type of vaccine used and the viral strains involved. It is clear that two vaccine doses produce a better response than a single dose,[14 15] and prior exposure to the circulating viral strains seems to increase the likelihood of response to the vaccine.[14 16] Protective antibody levels to individual viral strains following immunisation are reported in 29–75% of children on chemotherapy,[14–16] compared to around 70–90% of healthy children.[23 24] The results of this study are in keeping with an impaired response to vaccination in children with cancer, but nevertheless show a very useful response to immunisation. Similar impaired but useful response to influenza vaccine has been seen in some groups of adult patients receiving chemotherapy.[12 25 26]

By contrast with patients on treatment for cancer, children who have completed their treatment seem to show response rates more comparable with those expected in healthy controls.[16 18–21] For H3N2, the response was better in patients off treatment, although the difference was not statistically significant in the small number of patients off treatment in this study. The situation is different in bone marrow transplant recipients, a particularly high risk group with prolonged immunological impairment, who show no response at all until at least six months post-transplant.[27]

In our subgroup analysis we found no significant effect of tumour type (solid tumour versus ALL) on response to vaccination, but again a suggestion that response to H3N2 was better in solid tumour patients. As all the solid tumour patients were off treatment, the effect of tumour type could not be isolated from on versus off treatment. Children with solid tumours on treatment showed a significantly better response to A/New Jersey/8/76 than children being treated for ALL, although no difference was seen for A/Victoria/3/75.[16] Differences in response between patients with solid and haematological malignancies could

relate to the intrinsic disease associated immunosuppression of some haematological malignancies as well as the type and intensity of chemotherapy treatment. With the increased intensity of many chemotherapy regimes for solid tumours in recent years, it would be relevant to study response to a trivalent vaccine in a larger number of solid tumour patients currently on treatment.

Our study showed an interesting relation between age and response to immunisation for H1N1 and H3N2. In the two to three years prior to this study, H3N2 was the predominant circulating influenza strain, with limited H1N1. Influenza B had been largely absent.[28 29] The effect of age on response to H3N2 and H1N1 may therefore largely reflect previous exposure or "priming", with older children more likely to have been exposed. Gross found no difference in response to split virus H1N1 between 3–5 and 6–18 year olds where H1N1 had been absent from recent natural circulation,[23] but Wright *et al* showed a non-significant trend of better response with increasing age for H3N2 and B where these had been the previous circulating strains.[24] These results support a predominant effect of prior exposure rather than age alone on response.

One remaining area of uncertainty is whether an antibody level of 40, normally considered protective in healthy individuals, is actually protective in the immunocompromised host. In one study, 24% of unimmunised children with cancer developed proven influenza infection despite influenza virus titres of $\geq 1/32$,[6] but another study reported an incidence of flu like illness in only 10% of immunised children with leukaemia, compared to 46% of unimmunised children, suggesting that immunisation was associated with a reduced incidence of clinical infection.[13] The latter study showed a rise in antibody titres following immunisation, but post-immunisation titres were not discussed in relation to clinical infection in individual patients. The discrepancy between the studies could be explained if there is high rate of loss of protective antibody levels in patients receiving chemotherapy, a finding shown in the current study. If the latter explanation is correct, it may be that annual immunisation prior to the influenza season in children with cancer is of greater benefit than suggested by the change in the proportions of patients with protective antibody titres following immunisation, because it may reduce loss of protective immunity in some patients as well as facilitating new protective immunity in others. In one study of leukaemic children on and off treatment, antibody levels returned to baseline by 12 months following vaccination,[17] but in other children, mainly off treatment, levels were maintained at least until six months following vaccination,[21 22] suggesting that protection would be maintained during the highest risk time for influenza even if it is subsequently lost. Taken with previous data, our study suggests that the risk of influenza infection in children with cancer may be reduced substantially by immunisation.

Even though influenza usually runs a mild course in children with cancer, the occasional morbidity and mortality and high risk of interruption of potentially curative chemotherapy make it worth preventing. It seems sensible that the household contacts of such children and the hospital staff caring for them, as well as the children themselves, should receive annual immunisation against influenza,[5 10 30] although to our knowledge there is no direct evidence to support this approach. When proven influenza occurs despite immunisation, judicious use of zanamivir may reduce hospital admission rates and complications in high risk patients, but data in these groups are lacking.[31]

In conclusion, this study supports existing recommendations that all children with cancer receiving chemotherapy should receive annual immunisation with influenza vaccine. We suggest that their household contacts and appropriate hospital staff should also be immunised. Two doses of vaccine should be given at the first immunisation but data are needed on whether one or two doses of vaccine are required for second and subsequent vaccinations in patients on chemotherapy. Further data are also needed on the efficacy of the vaccine in children with solid tumours on treatment.

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Immunisation debates

Whether we like it or not "the media" are the most powerful influence on public opinion. There have been times when unbalanced reporting has done harm but recently (December 2000–January 2001) there has been evidence of a well-considered approach, at least to the subject of immunisation. The apparent success of group C meningococcal vaccination has been well publicised and MMR vaccination defended. Commenting on a recent fall in MMR acceptance rates, the *Times* concluded (January 5, 2001) that parents who refuse the vaccine for their children "shirk, for selfish reasons and on the basis of wholly inconclusive scientific research, a manifest social responsibility". Strong stuff, and probably unfair to many concerned parents, but at least fighting on the side of the angels.

Whenever questions are raised about immunisation there are fears about vaccine refusal, the great spectre being the example of pertussis in the early 1980s when disease rates soared after fears of vaccine-induced encephalopathy resulted in low acceptance rates. But questions must be asked. It may be irresponsible to endanger vaccination programmes on the basis of inadequate data but it is never irresponsible to ask sensible questions.

There is no doubt that parents do have their own thoughts about immunisation. In a national survey in the USA (Bruce G Gellin and colleagues. *Pediatrics* 2000;**106**:1097–102) 23% agreed that "children get more immunisations than are good for them", and 25% agreed, or expressed concern, that "too many immunisations could weaken the child's immune system". Could such concerns have any rational basis? Immunisation almost certainly has non-specific effects on immune function. Both measles and BCG immunisations probably boost immunity in non-specific as well as specific ways and so may other immunisations. In a randomised study in Germany (S Otto and colleagues. *Journal of Infection* 2000;41:172–5) babies who had their first immunisations at 3 months had more symptoms (cough, snuffles, restlessness, rash, or apparent pain) during their third month than did babies immunised at 2 months. The effects may be different in countries with high infection rates and high mortality. In Guinea-Bissau, West Africa (Ines Kristensen and colleagues. *BMJ* 2000;**321**:1435–8) measles and BCG immunisation improved non-specific immunity in young children but diphtheria, pertussis, and tetanus (DPT), and poliomyelitis immunisation appeared to impair it. This effect may reduce the effectiveness of DPT and polio immunisation and in this study DPT immunisation was associated with an increase in early childhood mortality. The *BMJ* published the paper together with a commentary which questioned the reliability of the results and an editorial which strongly defended current immunisation practice and hinted that there were those who thought that the paper should not have been published.

The television science guru Jacob Bronowski once wrote, "ask an impertinent question, and you are on the way to a pertinent answer". No subject, not even immunisation, is sacrosanct. Scaremongering is silly and irresponsible but questions have to be asked and debated. Evidence resolved the pertussis controversy and evidence has assuaged MMR fears. Parents' concerns must be faced and answered. Recent evidence suggests that the media can be an ally rather than an irritant in this process.

ARCHIVIST

Reduction in Functional Antibody Activity Against *Streptococcus pneumoniae* in Vaccinated Elderly Individuals Highly Correlates with Decreased IgG Antibody Avidity

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The pneumococcal polysaccharide vaccine is recommended as a means of preventing invasive disease in the elderly. We compared responses to the 23-valent polysaccharide vaccine in 46 previously unvaccinated, healthy, institutionalized elderly persons (mean age, 85.5 years) with those in 12 healthy younger adults (mean age, 37 years) by measuring prevaccination and postvaccination serum IgG antibody concentrations (by ELISA), functional antibody activity (by opsonophagocytosis), IgG antibody avidity, and passive protection in mice. Postvaccination IgG antibody concentrations for two serotypes (6B and 19F) of the five studied (4, 6B, 14, 19F, and 23F) were significantly lower in elderly than in younger adults; however, opsonophagocytic activity was significantly reduced for all serotypes in the elderly. Sera with reduced opsonophagocytic activity (titer, <64) correlated with low IgG antibody avidity and protected mice poorly against pneumococcal challenge. In elderly persons receiving polysaccharide vaccination, there was a significant reduction in the functionality of postvaccination antibodies, and this appeared to increase with advanced age.

Streptococcus pneumoniae is one of the leading causes of community-acquired pneumoniae and is associated with high morbidity and mortality among the elderly [1–3]. Elderly persons are at increased risk for pneumococcal pneumonia and bacteremia [1, 4, 5]. Vaccination with the 23-valent pneumococcal polysaccharide vaccine is recommended for persons ≥65 years of age, regardless of their immunocompetence status. To date, less than one-third of the eligible elderly population has been vaccinated [4, 6]. The estimated efficacy of pneumococcal vaccine in immunocompetent elderly persons (age range, 65–75 years) is 70%–78% [7, 8], and in all elderly persons, 44%–61% [9]. Although studies have provided data about the general efficacy of the vaccine, more accurate surrogates of protection are needed to evaluate immune status in the elderly after vaccination and to assist in developing recommendations for revaccination [4, 10].

See editorial response by Janoff and Rubins on pages 289–91.

The immune response to the polysaccharide vaccine in various age groups, including the elderly, has been studied previously [11–17]. Concentrations of antibody to pneumococcal capsular polysaccharides (PPSs) found in the elderly generally are thought to be similar to those in younger adults [12–14], although responses to certain polysaccharides may be reduced, with a more marked reduction in persons >85 years of age [14, 18]. In addition, elderly women may have lower responses than elderly men [14]. Data suggest that the protective effect of the pneumococcal vaccine diminishes with age [19]. A possible explanation is that anticapsular IgG antibodies of elderly persons may not be as effective in opsonizing pneumococci for phagocytosis. Studies of the functionality of the antibodies elicited by the 23-valent polysaccharide vaccine in older subjects [20] are very limited in number.

In the present study, titers of IgG antibody to PPSs and opsonophagocytic titers were determined prior to and following vaccination of institutionalized elderly persons and were compared with those in younger adult controls. In addition, antibody avidity and passive protection in mice were studied with use of selected sera from elderly persons who had high IgG antibody concentrations and low opsonophagocytic titers. This study contributes to the understanding of the immune response in elderly persons to the 23-valent pneumococcal

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Informed consent was obtained from all subjects. The protocols conformed to the guidelines established for human experimentation by the U.S. Department of Health and Human Services. The protocols used for animal experimentation were reviewed and approved by the Animal Use and Care Committee of the Baylor College of Medicine (Houston).

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polysaccharide vaccine and presents opsonophagocytosis as an immune status indicator that should be included in assessment of the immune response of elderly persons to polysaccharide vaccination.

Materials and Methods

Study group, vaccination, and sera collection. Forty-six elderly residents of a nursing home in Chicopee, Massachusetts, were vaccinated with the licensed 23-valent polysaccharide vaccine (Pnu-Immune 23; Lederle-Praxis-American Cyanamid, Pearl River, NY) as part of an epidemiological investigation of an outbreak of pneumonia in which parainfluenza virus and *S. pneumoniae* serotype 14 were implicated [21]. A dose of vaccine contained 25 μg of each of 23 pneumococcal polysaccharide types.

Ages of the elderly subjects ranged from 63 to 103 years (mean, 85.5 years); 39 were female and seven were male. The study group was stratified into three age groups: group 1, 63 through 79 years (n = 10); group 2, 80 through 89 years (n = 22); and group 3, ≥90 years (n = 14). Because the study population was primarily female, it was not stratified by gender. Paired sera were collected from all study participants. Sera designated as "pre" were collected within 3 days of vaccination; sera designated as "post" were collected 2–3 weeks following vaccination. Sera were stored at −70°C until tested. All participants were healthy at the time of vaccination; none was bedridden.

Control group. Twelve healthy younger adults (aged 22–46 years; mean age, 37.0 years)—an equal number of men and women—were used as controls. Paired sera obtained prior to and 4 weeks following vaccination with the Pnu-Immune 23 were collected and stored at −70°C.

ELISA IgG antibody concentrations. IgG antibody concentrations to PPSs from *S. pneumoniae* serotypes 4, 6B, 14, 19F, and 23F (abbreviated as PPS 4, PPS 6B, PPS 14, PPS 19F, and PPS 23F) were measured by a modified ELISA [22]. These serotypes are commonly isolated from patients with invasive disease and are associated with an increase in the frequency of resistance to drugs [3]. The 89SF standard reference serum (U.S. Food and Drug Administration, Bethesda, MD) was used to calculate serum antibody concentrations in micrograms per milliliter. Absorption of serum antibodies to the common cell-wall polysaccharide (CPS) was performed by incubation (for 30 minutes at room temperature) of diluted serum (1:50) in a solution of purified CPS (10 μg/mL; Statens Seruminstitut, Copenhagen). The substrate used was *o*-phenylenediamine dihydrochloride (Sigma, St. Louis).

Opsonophagocytosis. Functional antibody activity was measured in prevaccination and postvaccination sera by opsonophagocytosis (an antibody- and complement-dependent reaction), with use of differentiated HL-60 cells (granulocytes) as the effector cells [23]. HL-60 granulocytes can efficiently phagocytize and kill pneumococci, giving opsonophagocytic titers that highly correlate with those obtained with polymorphonuclear neutrophils from donors [23]. Opsonophagocytic titers were calculated for pneumococcal serotypes 4, 6B, 14, 19F, and 23F in a viability assay as the reciprocal of the serum dilution that had ≥50% killing by differentiated HL-60 cells, in comparison with antibody-free complement-rich controls (12.5% per well of 3- to 4-week rabbit serum; Pel-Freez, Brown Deer, WI). All pneumococcal strains used in this study were recent clinical isolates previously used as reference strains [23].

Antibody avidity determinations. The relative functional antibody avidity of selected postvaccination sera from elderly and younger adults with anticapsular IgG concentrations greater than or equal to a threshold concentration of 2 μg/mL and decreased functional antibody activity (below a threshold titer of 64) was compared with the avidity in postvaccination sera with ELISA levels of ≥2 μg/mL and functional activity of ≥64 in opsonophagocytic titers. Antibody avidity measures the relative strength of the antigen-antibody binding. Antibody avidity can affect the measurement of ELISA IgG concentrations used in the evaluation of vaccine-induced antibodies [24, 25].

Relative antibody avidity was determined by a modification of the method previously described by MacDonald et al. [26]. In brief, Immulon IV (Dynatech, Alexandria, Va) microtiter plates were coated with 10 μg/mL of each polysaccharide tested. A single predetermined serum concentration (from the linear portion of each serum ELISA IgG curve) was loaded onto each well in a 50-μL volume. Subsequently, 50 μL of a series of seven threefold dilutions of sodium thiocyanate (NaSCN; Sigma), a chaotropic compound that interferes with the antigen-antibody reaction, was added to each well, so that the final concentration ranged from 4 *M* to 0.05 *M*. Addition of NaSCN solution to the PPS-coated plates did not affect the amount of PPS bound to the plate. Plates were incubated at 37°C for 2 hours. The remainder of the assay was done following the antipneumococcal IgG-specific ELISA described above. The percentage reduction of the total absorbance (wave length, 460 nm) was calculated for each NaSCN concentration.

Passive protection in mice. The capacity of serum to passively protect mice against challenge with *S. pneumoniae* serogroup 4 was investigated in an adult mouse model with death as an endpoint [27]. Bacterial challenges were performed in groups of four outbred Swiss White mice (6–8 weeks old) with 10, 100, and 1,000 times the LD_{50}, 45 minutes after intraperitoneal injection of 0, 6, 18, 50, or 150 ng of IgG obtained by diluting the human sera to yield the desired dose in a final volume of 0.1 mL. One LD_{50} corresponded to 2–4 bacteria/mL. The number of surviving mice was recorded at 5 days after challenge.

Statistical analysis. Linear correlations were calculated with use of the Pearson's product moment correlation coefficient. Differences among groups of data were determined by

the Mann-Whitney rank-sum test, and those between pairs in 2-by-2 tables were determined by a two-tailed Fisher's exact test. Significance level was set at $P < .05$ for all tests. The opsonophagocytic titers and ELISA IgG antibody concentrations (μg/mL) were converted to a $\log_2$ base for statistical analysis. Opsonophagocytic titers <8 were reported as titers of 4 for calculation purposes. Single antibody avidity values were calculated as the weighted average of the NaSCN concentration able to reduce most of the ELISA IgG absorbance. Weights were assigned as the percent reduction of total absorbance for each serum at each NaSCN concentration. Statistical calculations were performed with use of SigmaStat software, version 1.0 (Jandel, San Rafael, CA), and Epi Info software, version 6.02 (Centers for Disease Control, Atlanta).

Results

IgG antibody detected by ELISA. Following vaccination, elderly subjects had significant increases in concentrations of IgG antibody to all serotypes tested. Comparison of the postvaccination IgG antibody concentrations of elderly adults with those of young adults revealed differences in the responses by age group. Table 1 gives the geometric mean concentrations (GMCs) of IgG and opsonophagocytic geometric mean titers (GMTs) for younger and elderly adults. IgG antibody GMCs in the elderly for serotypes 6B and 19F (GMCs of 5.1 and 5.8 μg/mL, respectively) were significantly lower than those in the young adult group (10.1 and 14.0 μg/mL, respectively).

Table 1. Geometric mean IgG PPS-specific serum antibody concentrations (μg/mL, per ELISA) and opsonophagocytic titers (reciprocal serum dilution) for elderly and young recipients of 23-valent pneumococcal polysaccharide vaccine.

		Value determined by indicated method, per age group			
		Young controls, aged 22–46 y (n = 12)		All elderly subjects, aged 63–103 y (n = 46)	
Streptococcus pneumoniae serotype	Serum specimen	ELISA	Opsono	ELISA	Opsono
4	Pre	0.8	10.4	0.8	5.2*
	Post	2.6	152.2	1.7	24.6*
6B	Pre	3.7	25.3	2.5	5.5*
	Post	10.1	352.1	5.1*	37.3*
14	Pre	0.8	19.0	2.6*	9.3*
	Post	10.0	304.4	10.0	76.6*
19F	Pre	5.2	20.1	2.9	7.1*
	Post	14.0	152.2	5.8*	28.7*
23F	Pre	2.6	7.5	2.5	7.9
	Post	6.2	71.5	4.8	22.0*

NOTE. Opsono = opsonophagocytosis; Post = postvaccination; PPS = pneumococcal capsular polysaccharide; Pre = prevaccination.

* Significant difference ($P < .05$) vs. values for the younger controls, by the Wilcoxon sample test (the Mann-Whitney rank-sum test was in agreement for the comparison between all younger adults and all elderly recipients for both ELISA IgG concentration and opsonophagocytic titer).

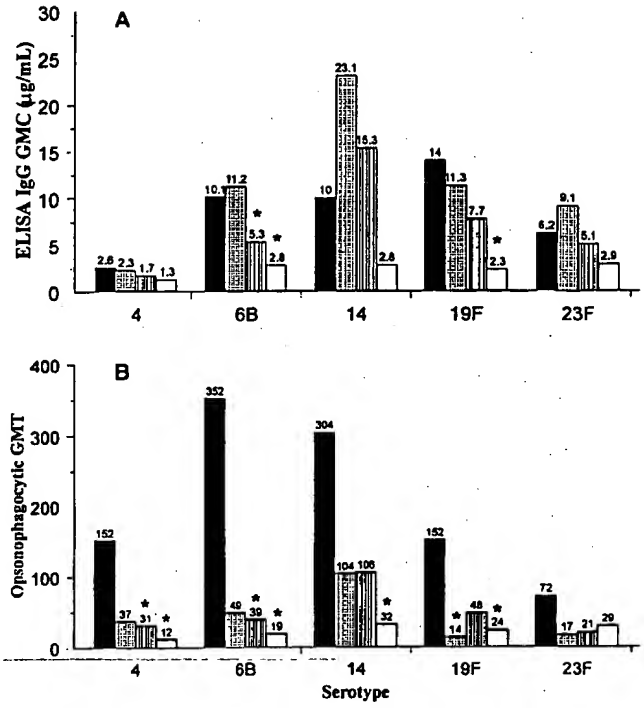

Figure 1. *A*, Geometric mean concentration (GMC; μg/mL) of IgG pneumococcal capsular polysaccharide–specific serum antibody, as determined by ELISA, for young controls (22–46 years; n = 12; *black bars*) and for elderly age groups 1 (63–79 years; n = 10; *dotted bars*), 2 (80–89 years; n = 22; *striped bars*), and 3 (≥90 years; n = 14; *open bars*) following vaccination with the 23-valent pneumococcal polysaccharide vaccine. *B*, Geometric mean opsonophagocytic titer (GMT; reciprocal serum dilution) for the young controls and elderly age groups shown in panel *A*, following vaccination. An asterisk designates a significant difference ($P < .05$) from values for the younger controls, per the Wilcoxon sample test.

Other significant differences in IgG concentrations in elderly and young adults following vaccination included lower GMCs against serotype 6B in elderly groups 2 and 3 (5.3 μg/mL and 2.8 μg/mL, respectively, vs. 10.1 μg/mL in young adults) and against serotype 19F in elderly group 3 (2.3 μg/mL, vs. 14.0 μg/mL in young adults) (figure 1A). Prevaccination ELISA IgG antibody concentrations were significantly different for serotype 14, when those in all elderly recipients were compared with those in younger adults.

The percentage of vaccinees attaining a twofold or higher rise in IgG antibody concentration was lower in the elderly than in young adults for serotypes 6B and 14 (table 2). However, no difference was observed in the numbers of elderly vs. young adult subjects with an IgG antibody concentration of ≥2 μg/mL following vaccination, except for serotype 19F (9 of 14 in elderly group 3 [age, ≥90 years] vs. 12 of 12 young adults).

Opsonophagocytic antibody activity. Far more striking than the IgG antibody differences given above were the significant reductions in postvaccination opsonophagocytic GMTs for the elderly vs. young adults. For all serotypes tested, there

Table 2. Percentage of elderly and young recipients of 23-valent pneumococcal polysaccharide vaccine with a twofold or higher rise in ELISA IgG concentration or a fourfold or higher rise in opsonophagocytic titer between prevaccination and postvaccination sera sampling.

	Percentage of recipients				Percentage of elderly recipients, per age group					
	Young, aged 22–46 y (n = 12)		All elderly, aged 63–103 y (n = 46)		Group 1: 63–79 y (n = 10)		Group 2: 80–89 y (n = 22)		Group 3: ≥90 y (n = 14)	
Streptococcus pneumoniae serotype	ELISA	Opsono	ELISA	Opsono	ELISA	Opsono	ELISA	Opsono	ELISA	Opsono
4	45	73	47	42	50	60	38	48	57	21*
6B	83	83	44*	57	70	50	43*	64	29*	50
14	92	75	56*	59	50	30	67	68	43*	50
19F	75	83	41	31*	30	20*	64	38*	14*	29*
23F	50	75	33	38*	30	40	32	38	36	36

NOTE. Opsono = opsonophagocytosis.

* Significant difference ($P < .05$) between young controls and elderly groups, per Fisher's exact two-tailed test. Prevaccination IgG concentrations (μg/mL) in the elderly were significantly elevated only for serotype 14.

was a significant reduction in opsonophagocytic GMTs for the elderly ($P < .05$) when compared with GMTs for young adults (table 1). Differences in opsonophagocytic GMTs were more evident as age increased: in group 1, differences were significant only for serotype 19F; in group 2, for serotypes 4 and 6B; and in group 3, for all serotypes except 23F (figure 1B).

Prevaccination opsonophagocytic titers of all elderly recipients were also significantly lower for all serotypes, except serotype 23F, when compared with those for younger adults (table 1). A significantly lower percentage of elderly vaccine recipients than young controls attained a fourfold or higher rise in opsonophagocytic titers (after vaccination) for serotypes 4, 19F, and 23F (table 2).

Serum IgG antibody concentrations ($\log_2$) in elderly and young adults had significant ($P < .05$) correlation coefficients of association (r values) with opsonophagocytic titers ($\log_2$) for all the serotypes tested (figure 2). However, postvaccination r values for serotypes 4, 6B, 19F, and 23F were lower for sera from elderly persons (r values ranged from .30 to .62) than for sera from young adults (r values ranged from .61 to .86). This reduction in correlation coefficients was due to a higher number of serum specimens from elderly persons who had IgG antibody concentrations of ≥2 μg/mL and opsonophagocytic titers of <64. The proportion of sera with opsonophagocytic titers of ≥64, as well as IgG antibody concentrations of ≥2 μg/mL, was significantly reduced in most elderly groups for serotype 6B (21%–45% in the elderly groups vs. 92% in young adults), serotype 19F (30%–42% for elderly groups vs. 83% in young adults), and serotype 14 (36% in elderly group 3 vs. 83% in young adults). To determine reasons for the differences in functional antibody activity observed between elderly and young adults, we further analyzed these sera to assess antibody avidity measurements and passive protection in mice.

Antibody avidity measurements. For the avidity assays performed in sera from elderly persons with low opsonophagocytic activity, low concentrations of NaSCN (range, 0.005 *M*–0.15 *M*) were able to reduce ELISA optical density by 85% (SD, ±13.2%). This suggested that the antibody measured by ELISA was of low avidity. In 37 serum assays that showed a discrepancy between IgG antibody concentration and opsonophagocytic titer, 34 (92%) of the specimens had low antibody avidity. In contrast, in sera with anticapsular antibody of ≥2 μg/mL (threshold IgG concentration) and a high opsonophagocytic titer (≥64 threshold titer), the reaction between IgG antibody and type-specific PPS was significantly inhibited only by higher NaSCN concentrations (≥0.44 *M*), suggesting the presence of high-avidity antibody in the sera. Figure 3A shows a significant correlation ($r = .76$, $P < .01$) between opsonophagocytic titer ($\log_2$) and the weighted average of NaSCN concentration. There was a significant correlation between ELISA IgG antibody concentration and the weighted average of NaSCN concentrations ($r = .36$, $P < .01$), although the r value was lower than that obtained with opsonophagocytosis (figure 3B). The association of low antibody avidity and low opsonophagocytic antibody activity was observed in sera from elderly and young adults.

Passive protection in mice. A high opsonophagocytic titer and high avidity for capsular PPS antigens were found to confer protection against pneumococcal challenge in mice. Thus, for example, serum 7023 (a specimen from an elderly subject that had high avidity and high opsonophagocytic activity) protected mice at all doses tested (table 3). In contrast, identical doses of IgG antibody from serum 7047, a specimen with low avidity and low opsonophagocytic activity from an elderly person, failed to protect even at the lowest inoculum of pneumococci. Sera with IgG antibody of intermediate avidity exhibited intermediate degrees of protection (data not shown). Because all sera tested had similar IgG antibody concentrations and yet conferred varying degrees of protection in mice, no association was observed between ELISA IgG antibody concentration and protection against death.

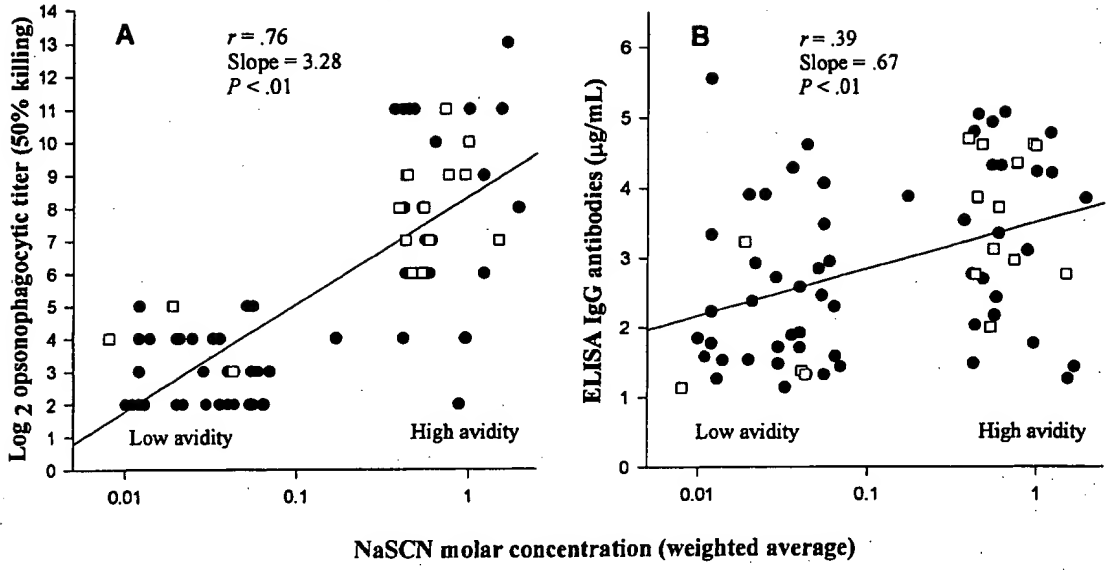

Figure 3. *A*, Correlation between the weighted average of the sodium thiocyanate (NaSCN) molar concentration yielding most of the reduction in ELISA IgG absorbance and the $\log_2$ opsonophagocytic titer. Results represent all serotypes (combined) in selected sera from elderly subjects (*black circles*) and young adults (*squares*). The line designates the linear regression. All sera with high antibody avidity also had high opsonophagocytic titers (≥64), except for sera from three elderly subjects that were tested against *Streptococcus pneumoniae* serogroup 4 and had low opsonophagocytic titers (<64) and high antibody avidity (weighted averages: 0.42 *M*, 0.88 *M*, and 0.96 *M* NaSCN). All serum samples shown had ELISA IgG antibody concentrations ≥2 μg/mL. *B*, Correlation between the weighted average of the NaSCN molar concentrations yielding most of the reduction in ELISA IgG absorbance and the ELISA IgG antibody concentration (μg/mL). Symbols are the same as in panel *A*. Sera with low antibody avidity (0.01 *M* to 0.1 *M* NaSCN) also had low opsonophagocytic titers (<64), regardless of the ELISA IgG antibody concentration. Sera with high antibody avidity (>0.4 *M* NaSCN) also had high opsonophagocytic titers (≥64).

passive-protection experiments in mice with use of *S. pneumoniae* serotype 4, in which 100% protection was achieved by administering a serum with high opsonophagocytic titer and antibody avidity; no protection was observed with a serum that had low opsonophagocytic titer and antibody avidity. Similar patterns of passive protection were observed for serotype 6B with sera from elderly and young adults in an infant mouse model of bacteremia (data not shown).

Differences between IgG antibody measured by ELISA and functional opsonophagocytic activity in sera from elderly persons appeared to be related to differences in the avidity of IgG for PPS. Low concentrations of NaSCN readily inhibited the interaction between PPSs and IgG antibody from sera with low opsonophagocytic activity, whereas much higher concentrations of NaSCN were needed to produce such inhibition in sera with high opsonophagocytic activity, such as antibodies against serotype 14.

Table 3. Passive protection capacity of sera from elderly persons with known ELISA IgG antibody concentration, opsonophagocytic titer, and antibody avidity against *Streptococcus pneumoniae* serotype 4.

Serum no.	Age (y) of patient	Dose of IgG antibody to serotype 4 (ng)	Percentage of mice surviving* after challenge with			Opsonic titer (50% killing)	ELISA IgG (μg/mL)	Antibody avidity: NaSCN†
			10 × LD_{50}	100 × LD_{50}	1,000 × LD_{50}			
7023	90	150	100 (4/4)	100 (4/4)	100 (4/4)	8,192	2.7	1.67
		50	100 (4/4)	100 (4/4)	100 (4/4)			
		18	100 (4/4)	100 (4/4)	100 (4/4)			
		6	100 (4/4)	100 (4/4)	100 (4/4)			
7047	74	150	25 (1/4)	0 (0/4)	0 (0/4)	4	2.4	0.013
		50	50 (2/4)	0 (0/4)	0 (0/4)			
		18	50 (2/4)	0 (0/4)	0 (0/4)			
		6	0 (0/4)	0 (0/4)	0 (0/4)			

NOTE. LD_{50} = lethal dose for 50% of adult mice (1 LD_{50} = 2–4 bacteria/mL).

* In parentheses is the no. of surviving mice/total no. of mice challenged.

† Molar concentration of sodium thyocyanate (NaSCN) necessary to yield an 85% reduction in ELISA IgG optical density.

In a study of older adults, Konradsen found no notable discrepancies between antibody concentration and avidity for various PPSs [29]; however, this finding may reflect the use of subjects 60–67 years of age, 20–40 years younger than the elderly subjects in our study. Similarly, Rubins et al. found no difference between elderly and younger adults in terms of serum antibody concentration and antibody avidity to serotype 14 [28]; however, as mentioned above, this serotype rarely elicits antibodies of low avidity in the elderly. Our study indicates that only nine of 46 elderly recipients had an opsonophagocytic titer <64 and an ELISA IgG antibody concentration ≥2 μg/mL against PPS 14. Neither one of these studies made a direct correlation between opsonophagocytic activity and antibody avidity.

In the immunogenicity studies reported to date, the elderly have responded equally to conjugated and unconjugated polysaccharides [30, 31]; however, the functional antibody response has not been addressed in these studies. If the poor functionality of antibodies to PPSs as humans age is caused by an adherent accessory cell deficiency for antigenic presentation in the spleen, as has been shown in mice [32], a vaccination strategy may not be able to overcome the defect. Reduced neutrophil function in the elderly (reduced activation of superoxide anions and increased level of apoptosis) could account for the increased risk for pneumococcal infections, regardless of vaccination status [33, 34].

In summary, this study highlights the importance of evaluating the IgG antibody response, as well as the functional antibody activity of the antibodies measured, especially in high-risk populations. The distinct reduction in functional IgG antibody activity in the elderly was more pronounced in those 80–89 years of age and ≥90 years of age. However, these age groups represent only 21% and 4.3%, respectively, of the target U.S. elderly population (32.4 million persons ≥65 years old) for the pneumococcal polysaccharide vaccine [35].

Thus, this study should not discourage the use of the 23-valent polysaccharide vaccine in the elderly until a better vaccination strategy is available. New and improved approaches to vaccination in the elderly should be considered.

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Antibody to Capsular Polysaccharide of *Streptococcus pneumoniae* at the Time of Hospital Admission for Pneumococcal Pneumonia

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IgG to capsular polysaccharide (CPS) of *Streptococcus pneumoniae* is thought to provide the greatest degree of protection against pneumococcal disease. Serum obtained at hospital admission from 14 (27%) of 51 patients with bacteremic pneumococcal pneumonia and 11 (37%) of 30 with nonbacteremic pneumococcal pneumonia contained IgG to CPS of the infecting serotype; these percentages are similar to the prevalence of IgG to CPS in a control population. However, when compared with antibody from healthy adults, this IgG had far less capacity to opsonize the infecting pneumococcal serotype for phagocytosis in vitro by normal human polymorphonuclear leukocytes or to protect mice against experimental challenge. Failure to opsonize correlated closely with failure to protect mice, and each of these parameters correlated well with poor avidity for CPS. Future vaccine studies may need to examine the functional capacity of antibodies as a surrogate for infection, in addition to measuring their concentration in serum.

For more than a century, humoral immunity has been recognized as the principal mechanism of defense against infection by *Streptococcus pneumoniae* [1–3]. IgG to capsular polysaccharides (CPS) is thought to provide the greatest degree of protection, and pneumococcal vaccine is administered to stimulate such antibody. However, a protective level of IgG has not been determined and, in light of recent studies showing that some older persons may generate poorly functional antibody [4, 5], it seems unlikely that such a level can be identified. It is not even certain whether, at the time of hospital admission, patients with pneumococcal pneumonia have antibody to the capsule of their infecting serotype. Use of a relatively insensitive agglutination technique in the preantibiotic era suggested that this antibody was not present [1]. In contrast, in the 1980s, the use of a relatively sensitive but nonspecific RIA [6] demonstrated levels of anticapsular antibody that were thought to be protective (>400 ng antibody N/mL) in nearly half of patients at hospital admission for pneumococcal pneumonia. This assay did not distinguish between nonprotective antibody to cell wall polysaccharide (CWPS) [7] and antibody to capsule; to our knowledge, such studies have not been repeated using the modern, sensitive yet specific ELISA [8, 9].

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We sought to determine whether, at the time of hospital admission, patients with pneumococcal pneumonia have antibody to the CPS of their infecting organism and, if they do, why this antibody is not protective. Our general method was to identify patients with pneumococcal pneumonia, retrieve the serum sample obtained from them at admission, serotype the infecting isolate, assay for antibody to the infecting CPS type by ELISA, and examine the functional activity in vitro and in vivo, if such antibody was present. We examined the in vitro activity by opsonization of an isolate of the same serotype for phagocytosis by human polymorphonuclear leukocytes (PMNL), and we examined the in vivo activity by the capacity to protect mice against challenge with this strain.

Methods

Subjects. Each day from 1 September 1996 through 31 March 1997 and from 1 September 1997 through 30 April 1999, one of the investigators reviewed bacterial culture results in the Microbiology Laboratory, Veterans Affairs Medical Center, Houston. Patients were initially considered for inclusion in this study if a culture of sputum or blood yielded *S. pneumoniae.* Patients were included (1) if they met case definitions (see below), (2) if ≥1 mL of serum that was obtained on the day of admission was available, and (3) if the infecting pneumococcal isolate was of a serotype contained in the 23-valent pneumococcal vaccine (the only types for which we regularly assay antibody). All sera were distributed to tubes in 0.5-mL aliquots. Sera and infecting bacterial isolates were stored at −70°C. Sera obtained on the day of admission are called "acute sera," and sera obtained 10 days to 12 weeks after the onset of pneumonia are called convalescent sera.

Bacteremic pneumococcal pneumonia. Patients with the diagnosis of bacteremic pneumococcal pneumonia had a clinical pre-

sentation consistent with pneumonia (generally including ≥1 of the following: subjective fever, cough, sputum production, or pleuritic chest pain), radiographic confirmation of a pulmonary infiltrate, and ≥1 blood culture that yielded *S. pneumoniae.*

Nonbacteremic pneumococcal pneumonia. Criteria for diagnosing nonbacteremic pneumococcal pneumonia were stringent in order to exclude patients for whom the diagnosis might be questionable. Such patients had all or nearly all of the following: clinical presentation suggestive of pneumonia with cough, sputum production, subjective fever or chills (or both), physical findings of pneumonia, and a distinct infiltrate on plain chest radiography. Examination by microscope of a gram-stained sputum sample (reviewed by D.M.M.) in every case showed ≥20 white blood cells per epithelial cell, with large numbers of gram-positive cocci in pairs and chains and no (or rare) other bacterial forms; sputum culture yielding *S. pneumoniae* and no other likely bacterial pathogens; and ≥1 negative and no positive blood cultures results before antibiotic therapy was begun. Many of these patients also had chest pain, documented fever in the hospital, and an elevated peripheral white blood cell count with increased numbers of bands.

Colonized subjects. Colonized subjects were persons from whose sputum *S. pneumoniae* was grown but who had no symptoms, signs, or radiographic changes suggestive of acute bacterial infection, who received no treatment for pneumococcal infection, and who showed no deterioration in the hospital that might have been consistent with an untreated bacterial disease. Such persons usually had a sputum sample cultured during evaluation of relatively stable lung lesions, such as pulmonary fibrosis, emphysema, or malignancy.

Control sera. Sera from 3 groups of healthy adults (control, positive control, and negative control subjects) were used individually or pooled. The control subjects were 34 healthy middle-aged adults who had participated in studies of pneumococcal vaccination in our laboratory. Sera obtained from the participants before and 4 weeks after they received a 23-valent pneumococcal vaccine had been stored at −70°C. To determine the prevalence of IgG before and after vaccination, we assayed these sera for IgG to 5 common CPS (CPS 3, 4, 6B, 19F, and 23F). The positive reference serum pool was constituted by combining equal volumes of postvaccination sera from healthy control subjects. Positive control subjects were 5 persons selected from the above-mentioned 34 on the basis of having postvaccination IgG levels ≥5 μg/mL to the 5 serotypes under study. Negative control subjects were healthy adults who were chosen because pre- and postvaccination sera contained no detectable IgG to the 5 pneumococcal serotypes cited above [10].

Serotyping. All pneumococcal isolates were typed, using procedures that are described in detail elsewhere [11], with antisera obtained from Statens Seruminstitut (Copenhagen). Coagglutination with *Staphylococcus aureus* was used to amplify the effect of the antiserum [11].

ELISA for antibody to CPS. Individual CPS (American Type Culture Collection, Manassas, VA) dissolved in PBS with 0.05% azide, 10–20 μg/mL, were incubated in wells of Immulon II plates (Dynatech, Chantilly, VA) for 5 h at 37°C and then incubated for 16 h at 4°C, as we have described elsewhere [12]. Before use, plates were blocked with PBS with 0.2% Tween 20 (PBST) for 1 h at 37°C and then washed 4 times with PBST. Sera were diluted 1:100 in PBS that contained 0.05% azide; isolated IgG suspensions from circulating immune complexes (CIC) were used undiluted. CWPS (10 μg/mL; Statens Seruminstitut) was added, and diluted sera and isolated IgG suspensions from CIC were incubated for 30 min at 4°C on a rocking platform, after which 2 3-fold dilutions were made from the already diluted, CWPS-adsorbed sera. Triplicate samples at each dilution were pipetted into wells coated with CPS and incubated at 37°C for 2 h, and wells were then washed 4 times with PBST.

IgG or CPS-reactive IgM was detected by the reaction of alkaline phosphatase–conjugated goat anti-human IgG or IgM and its substrate (Sigma, St. Louis). Each ELISA plate included 6 dilutions of a positive laboratory reference standard serum with a known concentration of IgG or IgM to a specific CPS based on the common reference serum 89-SF (provided by the Center for Biologics Evaluation and Research, Rockville, MD) as well as a negative laboratory reference serum that contained no IgG or IgM to the individual CPS under study [10]. Sera that yielded a result equal to or less than the negative reference serum were read as zero, although because of variation in the baseline and the asymptotic shape of the positive reference standard curve, a low level of IgG (generally <0.5 μg/mL) might, on occasion, appear to be present. Optical density was recorded by an automated reader (Dynatech).

After the antibody assays described in this study had been completed, we became aware of the possibility that adsorption with CPS from type 22F together with CWPS might remove cross-reacting IgG more completely than adsorption with CWPS alone. Sera that contained antibody were studied again after adsorption in this fashion; measured levels of IgG declined by ≤30%, and in no case was a serum that was regarded as containing antibody to the CPS of the admitting strain converted to one that seemed to lack antibody.

Separation of immune complexes. To precipitate CIC [13], we added 1 mL of serum to 1 mL of 12.5% polyethylene glycol in 0.1 *M* borate buffer (pH 8.4) and added 3 mL of distilled water to bring the volume to 5 mL. The resulting solution was maintained at 4°C for 90 min, after which the pellet was separated by centrifugation and washed three times with 2 mL of 2.5% polyethylene glycol in 0.1 *M* borate buffer. Antibody was dissociated by resuspending the precipitate in 1 mL of 0.1 *M* borate buffer (pH 10.4). IgG from the dissociated complexes was purified using 1-mL affinity columns (HiTrap protein A; Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's directions.

Flow cytometry assay of phagocytosis. Pneumococcal colonies were grown for 15 h at 37°C in 5% CO_2 on blood agar plates and then scraped from the surface of the plates and suspended in Hanks' balanced salt solution without Ca^{2+} or Mg^{2+} (HBSS), yielding ~10^9 cfu/mL. To count colony-forming units, we serially diluted and plated an aliquot. The suspension was washed twice, incubated at 56°C for 30 min to inactivate autolysin, washed again, and resuspended in 0.1 *M* $NaHCO_3$ buffer (pH 9). Fluoroscein-isothiocyanate (FITC; 1 mg/mL; Sigma) was added, and the suspension was incubated on a shaking platform in the dark at 4°C for 18 h. FITC-labeled bacteria were washed 5 times and resuspended in HBSS to yield 10^8 cfu/mL; these bacteria were dispensed to tubes wrapped in aluminum foil and stored at −20°C for use within 3–4 months.

PMNL were isolated from whole blood of healthy adults by sedimentation in Histopaque 1119 and 1077 (Sigma). Red blood cells were lysed by brief exposure to 0.2% saline, after which PMNL were

resuspended in HBSS with 0.1% gelatin (GHBSS) to 5×10^6 cells/mL.

Bacteria were opsonized as follows. Each reaction mixture contained serum, which was diluted to yield 2 μg/mL IgG to the relevant serotype, 5×10^6 cfu bacteria, and 6% complement-rich guinea pig serum in a final volume of 225 μL. If the IgG concentration of the original serum was below a level that can be diluted to 2 μg/mL in the final mixture, a lower concentration (but not ≥1 μg/mL) was used. This mix was incubated for 30 min in a vigorously shaking water bath at 37°C. The reaction was stopped by adding 1 mL of ice-cold HBSS. Opsonized bacteria were washed once and resuspended in 100 μL of GHBSS containing Ca^{2+} and Mg^{2+}.

For the phagocytosis assay, flow cytometry was used to quantitate cell-associated bacteria by a modification of a previously reported method that included experiments showing that cell-associated bacteria were largely taken up by the phagocytic cells [14]. GHBSS (100 μL) with Ca^{2+} and Mg^{2+} and containing 5×10^5 PMNL were added to the opsonized bacteria to yield a ratio of 10 bacteria to 1 PMNL. The bacteria-PMNL suspension was incubated for 30 min in a vigorously shaking water bath at 37°C. The reaction was stopped with 1 mL of ice-cold GHBSS, followed by a wash, and the pellet was resuspended in 400 μL of GHBSS. Fluorescein from surface-adherent bacteria was quenched by adding 100 μL of 0.1% trypan blue (pH 7.2) and incubating for 10 min at room temperature. The suspension was washed twice with 5 mL of GHBSS and resuspended in 350 μL of GHBSS with 1% paraformaldehyde (pH 7.35). Uptake of fluorescein-labeled pneumococci by PMNL was measured by flow cytometry (FACSII; Becton Dickinson, Sparks, MD).

Duplicate samples of 10,000 cells each were analyzed. PMNL were gated according to their characteristic light-scattering pattern. Log_{10} fluorescein intensity (FI) was plotted against cell number. Total FI for each PMNL sample was calculated on the basis of the total number of labeled PMNL and the mean FI of each PMNL. Data are reported as the opsonic index, which is the total FI generated by patient serum divided by the total FI generated by the positive reference serum pool (expressed as a percentage). Opsonic index was related to functional activity by stratifying data on the basis of a cut-off opsonic index value of 50, such that indices <50 are considered as poorly functional.

Each phagocytosis experiment included 4 negative controls: (1) a negative laboratory reference serum that lacked IgG to the relevant CPS being studied, (2) a sample in which diluted serum was replaced with HBSS, (3) a sample in which both diluted serum and guinea pig serum were replaced with HBSS, and (4) a sample in which labeled bacteria were replaced with HBSS. These negative controls were included to ensure that (in the order they are listed) (1) phagocytosis was not due to immune components in serum other than specific IgG to CPS, (2) phagocytosis was not attributable to the effect of guinea pig serum alone, (3) serum IgG and complement were both needed for optimal phagocytosis, and (4) the fluorescein signal was from labeled bacteria. These controls consistently showed low-level activity: (no. 4 above) < (no. 3), and (no. 2) < (no. 1) ≤ opsonic index of 10. Whenever possible, sera from 1 patient in each group and 1 control subject were studied concurrently. Acute and convalescent sera were always studied concurrently.

Mouse protection. Outbred Swiss white mice, weighing 18–25 g, were housed at an ambient temperature of 22°C in a room that was lighted for 12 h each day. Sera were diluted in saline to yield a range of concentrations of anticapsular IgG, and 0.1-mL volumes were injected intraperitoneally into groups of 4–5 animals [15]. For each experiment, groups of mice received diluted acute serum and, if available, convalescent serum from ≥1 patients and the positive reference serum pool with equivalent concentrations of anticapsular IgG. Control animals received 0.1 mL of saline. Absorption of particulate or dissolved material from the peritoneal cavity is rapid and complete [16]. Forty-five minutes later, mice were injected intraperitoneally with 10 LD_{50} of the relevant pneumococcal serotype, which was obtained by thawing a well-characterized frozen stock and serially diluting it in saline. cfu were verified by dilution and plating after injection. Mice were observed daily for 7 days, after which no deaths occurred. The protective index was defined as the number of mice that survived after receiving the highest dose of IgG from a patient serum divided by the number of mice that survived at the same dose of IgG from the positive reference serum pool expressed as a percentage.

In phagocytosis and mouse protection experiments, a representative isolate of the same serotype as the infecting one was used to insure uniformity of data. In addition, because we have not been able to construct a column that successfully purifies anticapsular antibody, we did not use isolated IgG to CPS; rather, in each experiment, whole serum was adjusted by dilution to achieve the desired concentration of anti-CPS IgG. As a result, the possible contribution of other antibodies to phagocytosis in vitro and/or to protection of mice after pneumococcal challenge can not be excluded. However, in our assay, the absence of anticapsular IgG results in the failure to opsonize for phagocytosis, as shown above, or to protect mice, factors which seems to argue against an important protective role for other antibodies.

Determination of avidity constant by inhibition ELISA. Sera were diluted to yield 0.3 μg/mL IgG to the relevant CPS, a concentration which gives a reading of ~1 optical density unit by ELISA. Diluted sera were incubated with CWPS to remove cross-reacting IgG. CPS was serially diluted to give concentrations ranging from 0.08 to 60 μg/mL and added to diluted sera; these mixtures were then incubated at 4°C on a rocking platform for 60 min. Diluted sera without CPS were studied to determine total free IgG. After this step, ELISA was done as described above except that serum incubation was limited to 1 h in order to minimize the disturbance of the solution-phase equilibrium, which may be caused by the binding of IgG to solid-phase CPS. Every ELISA plate included a laboratory standard with a known concentration of IgG to the relevant CPS.

IgG that was bound to CPS in solution was determined by subtracting free IgG, which was detected by ELISA (IgG bound to solid-phase CPS) from the total IgG as measured in sample without the inclusion of CPS. A Michaelis-Menten plot was constructed, with CPS (μg/mL) shown on the *x*-axis and bound IgG in solution (expressed in optical density units) on the *y*-axis. The association between anticapsular antibody and CPS substrate was determined by calculating the binding constant *(Km)* and the saturation rate at which antibody binds CPS (V_{max}), according to the equation $V = [V_{max}(S)]/[(S) + Km]$, where V is the reaction rate and S is the concentration of CPS. Values of Km and V_{max} were calculated by nonlinear regression data analysis (Grafit; Erithacus Software,

Table 1. Presence of anticapsular IgG or circulating immune complexes (CIC) in serum from patients infected or colonized with *Streptococcus pneumoniae.*

Complex, serum	*n*	Patients		
		Bacteremic	Nonbacteremic	Colonized
Anti-CPS IgG, acute	100	14/51 (27)	11/30 (37)	8/19 (42)
CIC, acute	34	1/18 (6)	0/9 (0)	1/7 (14)
Anti-CPS IgG, convalescent	30	8/13 (62)	9/13 (69)	2/4 (50)

NOTE. Data are no. positive/no. studied (%). Acute serum, serum obtained at admission; convalescent serum, serum obtained 10 days to 12 weeks after the onset of pneumonia. CPS, capsular polysaccharide of the infecting serotype.

Staines, UK). The unit of V_{max} was converted from optical density units to micromoles per milliliter per minute on the basis of the positive standard, the molecular mass of IgG (150,000 daltons), and the reaction time of 60 min. The avidity constant is defined as the ratio V_{max} to *Km,* reported as (μmol of bound IgG in solution)/(μg of CPS)/min.

Statistics. SAS software (version 6.12; SAS, Cary, NC) was used for χ^2 and regression analyses. Analysis for continuous variables using Student's *t* test was performed with Excel software (version 5.0; Microsoft, Redmond, WA).

Results

Antibody to CPS. Serum from the day of admission ("acute serum") was available from 100 patients: 51 with bacteremic pneumococcal pneumonia, 30 with nonbacteremic pneumococcal pneumonia, and 19 who were colonized with *S. pneumoniae.* Most patients lacked detectable IgG to CPS of the same type as their infecting pneumococcus; only 14 (27%) of 51 bacteremic and 11 (37%) of 30 nonbacteremic patients had such IgG at the time of admission, as did 8 (42%) of 19 who were colonized (table 1). We determined the prevalence of IgG to 5 common pneumococcal CPS (3, 4, 6B, 19F, and 23F) in sera from 34 healthy, age-matched control subjects. Of 170 possible reactions (5 × 34), 48 (28%) were positive, a percentage similar to that observed in our patients at the time they were admitted with pneumococcal infection or colonization ($P = .5$, $2 \times 4\chi^2$ comparison).

A second serum sample was obtained 10 days to 12 weeks after admission ("convalescent serum") from 30 subjects (13 bacteremic, 13 nonbacteremic, and 4 colonized). At this point, 19 (63%) of the 30 subjects had IgG to CPS of the infecting serotype (table 1); this proportion was similar to the 61% of reactions that were positive in control subjects after pneumococcal vaccination.

At 0.9 μg/mL, the geometric mean IgG was lowest in acute sera of bacteremic patients, compared with 1.2 and 1.5 μg/mL in nonbacteremic and colonized subjects, respectively (differences not significant). The geometric mean in convalescent sera increased ~3-fold in bacteremic patients (from 0.9 to 2.5 μg/mL, $P = .34$) and nonbacteremic patients (from 1.2 to 3.8 μg/mL, $P = .05$), while remaining unchanged in colonized subjects (1.5 vs. 1.5 μg/mL, $P = .39$). When geometric mean IgG in acute and convalescent sera was calculated using only data from paired sera, similar results were obtained (figure 1).

Detection of antibody in CIC. CIC were isolated and dissociated from a convenience sample of 34 acute sera (18 patients with bacteremic pneumonia, 9 with nonbacteremic pneumonia, and 7 who were colonized). IgG to CPS of the infecting strain was found by ELISA in CIC from 2 serum samples (1 from a patient with bacteremic pneumonia and the other from a colonized subject). These results (table 1) suggest that the failure to detect IgG in serum signifies a true absence of such antibody, although they do not exclude the possibility that IgG is bound to pneumococci in the lungs without release of complexes to the circulation.

Opsonophagocytosis. Pilot studies showed linear dose-response curves relating the uptake of pneumococci to opsonizing concentrations of IgG from 1 to 10 μg/mL (obtained by dilution of individual sera) or to bacteria:PMNL ratios of 10:1 to 100:1 (using bacteria of the same serotype as the infecting organism). Accordingly, in subsequent experiments, opsonization was carried out with individual patient sera and the positive reference serum diluted to yield a final concentration of 2 μg/mL anticapsular IgG, with the exception of sera that contained <2 μg/mL of anticapsular IgG, as explained in Methods. Phagocytosis was studied at a ratio of 10 bacteria to 1 PMNL.

Results, reported as opsonic indices (defined in Methods), showed that serum antibody present at admission for pneumococcal pneumonia was poorly opsonic for the infecting serotype compared with that from colonized subjects, the positive reference serum pool, or individual positive control sera (stud-

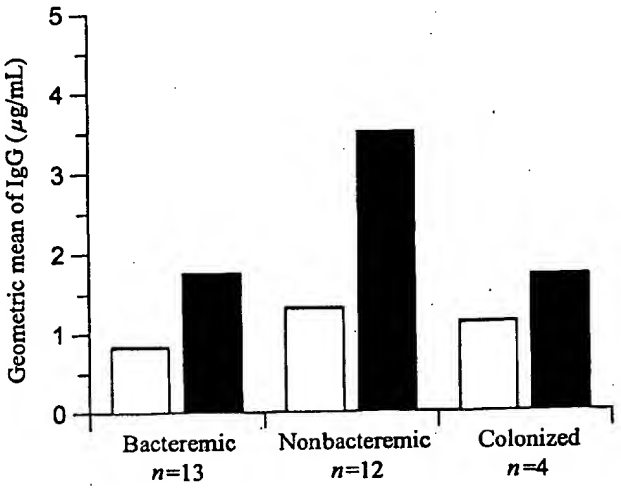

Figure 1. Geometric mean IgG to capsular polysaccharide of the infecting serotype was determined for bacteremic or nonbacteremic pneumococcal pneumonia patients and for colonized patients. Results are shown only for patients from whom both acute serum (□) and convalescent serum (■) were available (classes of sera are defined in Methods).

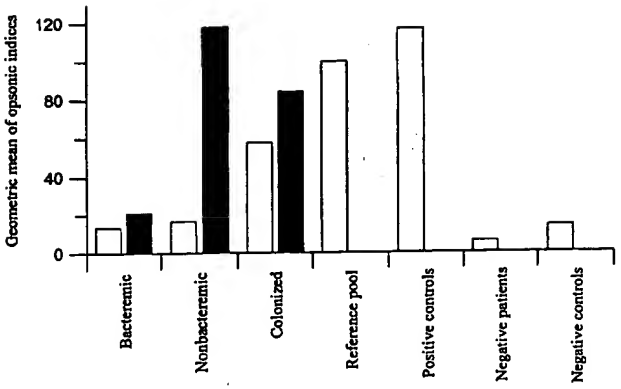

Figure 2. Geometric means of opsonic indices (defined in Methods) are shown for study subjects. Pneumonia patients (bacteremic and nonbacteremic) and healthy control subjects lacked measurable IgG to the capsular polysaccharide. □, Acute serum; ■, convalescent serum (defined in Methods).

ied only for serotypes 4 and 23F). The geometric mean opsonic index for all acute sera studied was 16.7 for bacteremic and 13.4 for nonbacteremic pneumococcal pneumonia patients, compared with 58.0 for colonized subjects, 100 (by definition) for the positive reference serum pool, and 117 for positive control subjects (figure 2). The differences are significant for bacteremic or nonbacteremic acute sera versus positive control sera ($P < .005$) but not for colonized subjects ($P = .45$).

The finding that the geometric mean opsonic index for individual positive control sera closely resembled that of the positive reference pool serum validates the averaging effect that resulted from pooling sera. The geometric mean opsonic index was 6 for acute sera from 10 pneumonia patients who lacked antibody to their infecting serotype (serotypes 4 and 23F were used for these experiments), and the index was 14 for acute sera from 5 healthy adults (negative controls) who had no detectable IgG to CPS 4 and 23F. These indices were significantly lower than those for positive control subjects ($P < .001$; figure 2), and confirm that sera lacking antibody to CPS failed to opsonize for phagocytosis

In convalescence, opsonization by serum from bacteremic patients remained low (geometric mean opsonic index, 21.3), while the geometric mean opsonic index from nonbacteremic patients rose to 118.2, a significant increase over that for acute sera from these same subjects ($P < .005$), and exceeded values for convalescent sera of bacteremic patients ($P = .12$). The geometric mean opsonic index in follow-up sera from colonized subjects rose to 84.8, approaching that for the positive reference serum pool (figure 2).

When results were stratified into low (<50) or normal (≥50) opsonic indices, acute sera from 11 of 12 bacteremic, 9 of 10 nonbacteremic patients, and 3 of 5 colonized subjects had low functional activity (table 2). Among the control subjects that we studied, 10 of 10 IgG-negative patients and 5 of 5 negative control subjects had minimal opsonic activity, whereas 10 of 10 individual positive control subjects, selected because they had IgG to CPS 4 and 23F, had normal opsonic activity. The differences in the proportions of persons whose sera had low or normal functional activity are significant when comparing positive control subjects and bacteremic or nonbacteremic patients or colonized subjects ($P < .05$ for each comparison). Antibody detected in convalescent sera was likely to be more functional, showing an opsonic index ≥50 in 3 of 6 bacteremic, 7 of 8 nonbacteremic, and 2 of 2 colonized subjects (table 2). The increase in functional activity from 1 of 10 acute sera to 7 of 8 convalescent sera in nonbacteremic patients was significant ($P < .005$).

Mouse protection. The capacity of anticapsular antibody in patient sera to protect mice against challenge with mouse-virulent serotypes (types 3, 4, and 8 in this study) was then examined in vivo. In each experiment, patient sera and the positive reference serum pool were adjusted by dilution in saline to deliver a range of desired doses of IgG that varied with the serotype. In 2 separate experiments (figure 3), 11 ng of IgG to type 4 CPS from serum of a healthy adult nearly completely protected mice against challenge with *S. pneumoniae* type 4, and 33 ng of IgG was fully protective. In contrast, 33 ng of IgG from acute serum of a patient with type 4 bacteremic infection protected only 2 of 10 mice.

Figure 4 shows data from an experiment in which 150 ng of IgG to CPS type 3 from acute sera for 2 bacteremic patients with type 3 infection (the highest dose that could be administered in 0.1 mL of undiluted serum) provided no protection against type 3 challenge. In contrast, lower amounts of IgG from the positive reference serum pool or from a nonbacteremic patient's acute or convalescent serum were partially protective. In this experiment, the positive reference serum pool was most protective, followed by the convalescent serum of the nonbacteremic patient and finally the acute serum of the same nonbacteremic patient. All experiments were repeated one or more times, limited in some instances by the amount of serum available. Similar results were obtained in other experiments. Statistical analyses were not performed on the protective index data because of the few data points for each category.

Correlation of opsonic and protective indices. Passive protection against lethal challenge correlated well with a high se-

Table 2. Opsonic indices (OI; defined in Methods) of serum from patients with pneumococcal pneumonia and colonized subjects.

Patients or subjects	Acute serum			Convalescent serum		
	n	OI <50	OI ≥50	*n*	OI <50	OI ≥50
Bacteremic	12	11 (92)	1 (8)	6	3 (50)	3 (50)
Nonbacteremic	10	9 (90)	1 (10)	8	1 (12)	7 (88)
Colonized	5	3 (60)	2 (40)	2	0 (0)	2 (100)

NOTE. Data are no. (%) of patients. Acute serum, serum obtained at admission; convalescent serum, serum obtained 10 days to 12 weeks after the onset of pneumonia.

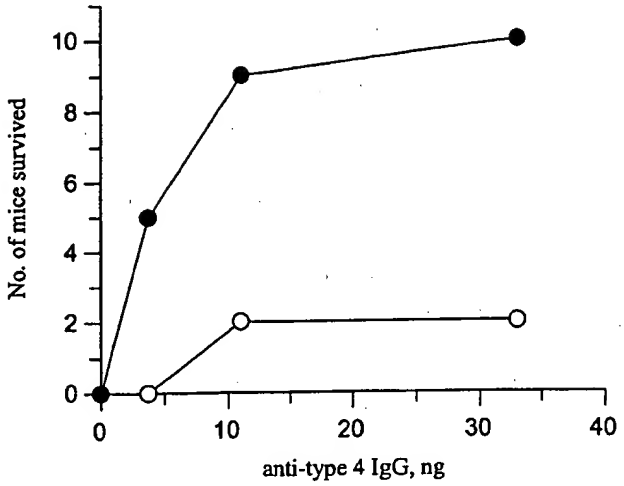

Figure 3. Protection of mice against challenge with *Streptococcus pneumoniae* type 4 by increasing admitting doses of anti-capsular type 4 IgG from the serum of a patient with type 4 bacteremic infection (○) or from serum of a healthy adult (●). Shown are cumulative results of 2 experiments.

rum opsonic index. When results of all experiments were included, a significant correlation ($r = .73$, $P < .001$) was observed between serum opsonic and protective indices (figure 5).

Correlation of antibody avidity and functional capacity. Antibody avidity as measured in vitro by an inhibition ELISA assay generally correlated well with functional activity as measured in vitro and in vivo. Sera that opsonized pneumococci well for phagocytosis or protected mice against experimental challenge (or both) had high-avidity anticapsular IgG, whereas those that did not opsonize and/or protect generally had low-avidity IgG. For *S. pneumoniae* serotype 3, which was associated with the highest number of patient sera containing IgG, correlation plots between antibody avidity constant and serum opsonic index, serum opsonic index and serum protective index, or antibody avidity constant and serum protective index show high correlations ($r = .90$, $P < .01$; $r = .93$, $P < .005$; $r = .72$, $P < .1$, respectively; figure 6). For only 2 sera in this study, in vitro phagocytosis did not correlate well with mouse protection. Both were from nonbacteremic patients with *S. pneumoniae* type 4 infection, and in both instances the serum protected mice but did not stimulate phagocytosis in vitro. One of these serum samples was available for avidity testing and was found to exhibit good avidity in vitro; the other sample was depleted during mouse protection studies, before avidity testing was done.

IgM levels. To determine the potential contribution of IgM to these results, we studied acute and convalescent sera from all subjects who were infected by or colonized with *S. pneumoniae* types 3 or 4. In the acute sera, 7 had IgG to the relevant serotype, and of them, 2 had IgM. One of these subjects was bacteremic, and the serum did not opsonize; the other subject was colonized and his serum showed opsonic activity. Of 8 without IgG, none had IgM. Of 8 patients from whom convalescent serum could be obtained, 1 each who had or did not have IgG also had IgM. One of these was bacteremic, and the other was nonbacteremic; neither serum opsonized the infecting organism. These results suggested that IgM contributed imperceptibly to the observed results.

Discussion

Pneumococcal pneumonia remains a common and serious medical problem, especially among elderly persons and people with underlying diseases [1, 17–19]. Despite appropriate antibiotic therapy, mortality from bacteremic pneumococcal pneumonia in hospitalized patients approaches 25% [17–19], and there is fear that the rates of complications and death may increase with increasing resistance of *S. pneumoniae* to commonly available antibiotics. These factors have helped to rekindle interest in immunity to pneumococcal disease.

Throughout the past century, investigations of antipneumococcal immunity focused on antibody to CPS [1–3]; however, in the past decade, research efforts have broadened to include a possible role for antibody to other surface constituents as well [20–23]. Advances in methodology, first with the application of RIA [6] and later of ELISA [8, 9], made it possible

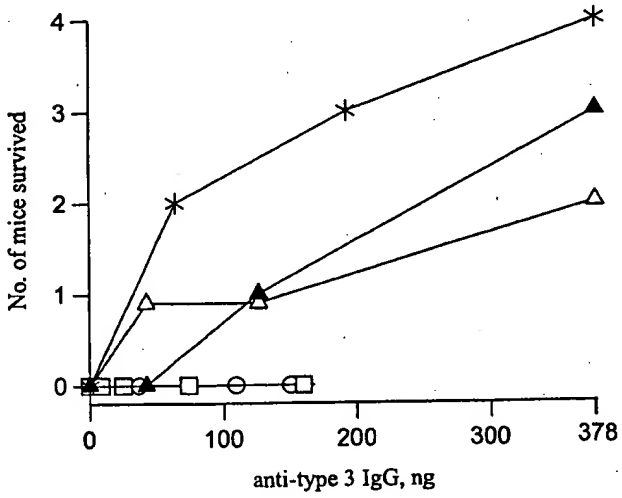

Figure 4. Protection of mice against challenge with *Streptococcus pneumoniae* type 3 by increasing doses of anti-capsular type 3 IgG from serum of patients with type 3 infection at time of admission *(open symbols)* or in convalescence *(closed symbols)*. ○ Bacteremic patient 1; □, bacteremic patient 2; △, acute nonbacteremic patient 1; ▲, convalescent nonbacteremic patient 1. * Positive reference serum pool. Bacteremic patients 1 and 2 did not survive, so convalescent sera (defined in Methods) were not available. In these experiments, there were 4 mice per group.

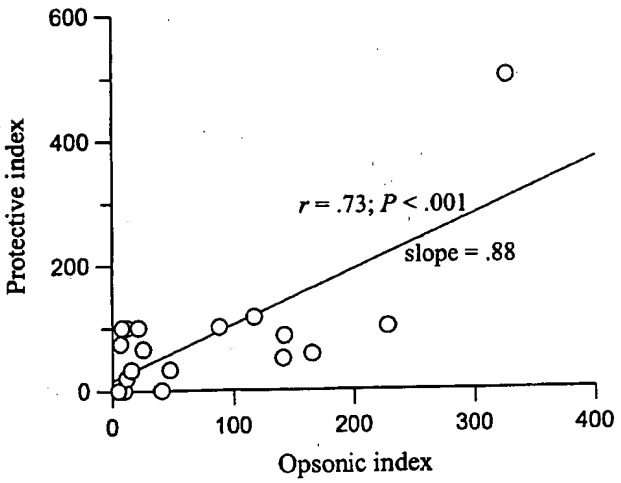

Figure 5. Correlation of protective and opsonic indices (defined in Methods) is shown for all individual sera studied. Linear regression analysis revealed $r = .73$, $P < .001$.

to quantitate antibody, whether acquired naturally or after administration of pneumococcal vaccine.

The observation that elderly persons and people with underlying diseases remain susceptible to pneumococcal disease even after vaccination [24, 25] led to the hypothesis that antibody levels in such populations were not as high or that the antibody did not function as well. An early study from our laboratory [26] showed that after vaccination, older men with chronic lung disease have lower levels of antibody than do healthy young adults. Sankilampi et al. [27] and Musher et al. [28] reported that the responses of very healthy elderly adults to pneumococcal vaccine are similar to or, in the case of certain polysaccharides, slightly lower than the responses of younger persons. Older adults who are not preselected for excellent health and vigor and who may have a variety of medical conditions associated with aging have lower postvaccination levels of IgG than do younger adults [4, 5]. More important, such persons may generate antibody that is poorly functional, as shown in vitro by poor opsonization for phagocytosis by tissue-cultured cells and in vivo by reduced protection of mice against pneumococcal challenge [4, 5].

By focusing on patients who actually have pneumococcal pneumonia, the present study enables us to examine more directly the degree to which preexisting antibody to the CPS of an infecting organism might protect against the development of infection. We found that at the time of admission to the hospital, most patients with pneumococcal pneumonia lack antibody to the CPS of their infecting serotype. When less sensitive techniques were used in the preantibiotic era, antibody was said to be absent at the time pneumonia is diagnosed; in contrast, we found that about one-third of patients have such antibody. Of interest, the prevalence of antibody in our patients was about the same as it is in the population at large [12]. Of even greater interest was the finding that anti-CPS IgG present at admission had diminished functional activity. Unlike sera from healthy age-matched adults, acute sera from patients, when adjusted by dilution to yield a fixed amount of IgG to CPS, failed to opsonize the infecting pneumococcal serotype effectively in vitro in nearly every instance.

Most pneumococcal types are not virulent for mice (virulence defined as an LD of $<10^5$ cfu/mL). Using strains of *S. pneumoniae* types 3, 4, and 8 that were isolated from patients and are virulent for mice, we found that serum containing anti-CPS IgG failed to protect mice against challenge in vivo. Results of an in vitro test of avidity suggested that the nonprotective antibody fails to bind CPS avidly. Taken together, these results suggest that patients are prone to pneumococcal infection either because they lack IgG to CPS or because, if such antibody is present, it functions poorly.

In general, our patients exhibited increases in IgG to type-specific CPS after infection. The proportion of subjects with IgG-to-CPS-of-their-infecting-serotype increased from about one-third to about two-thirds, again similar to the proportion of normal subjects who developed antibody after pneumococcal vaccination. Antibody produced in response to infection was more likely to be functional, at least in patients who did not have bacteremia. Of the small sample of subjects whose convalescent serum was studied, 88% of nonbacteremic patients and 50% of bacteremic patients had functional antibody.

Generation of poorly functional antibody is not exclusive to older, diseased individuals. Nahm et al. [29] have shown that pneumococcal vaccination stimulates the appearance of IgG that is cross-reactive (i.e., reactive with related, nonvaccine serotypes) and that such cross-reactive IgG has less opsonic activity than homologous IgG. Usinger and Lucas [30] showed that because of the polymorphic nature of IgG_2 responses to CPS, healthy young adults who receive pneumococcal vaccine generate IgG with a range of avidity for the polysaccharide antigen. Several important problems remain to be addressed, including whether persons who generate poorly functional antibody to one polysaccharide do so to all polysaccharides, at what point and how (at a molecular level) the change from functional to nonfunctional antibody takes place, and whether presentation of antigen in a different form (e.g., as a protein conjugate) will stimulate production of better functioning antibody.

The results of the present study provide further insights into the nature of immunity to pneumococcal infection. In an early study, Musher et al. [7] reported a direct relation between the IgG level measured by ELISA and opsonization of pneumococci or protection of experimental animals. In addition, Vidarsson et al. [31] showed a high degree of correlation between the concentration of IgG to CPS and opsonizing capacity; the findings of both these reports were based on serum from healthy young adults. Usinger and Lucas [30] show good correlation

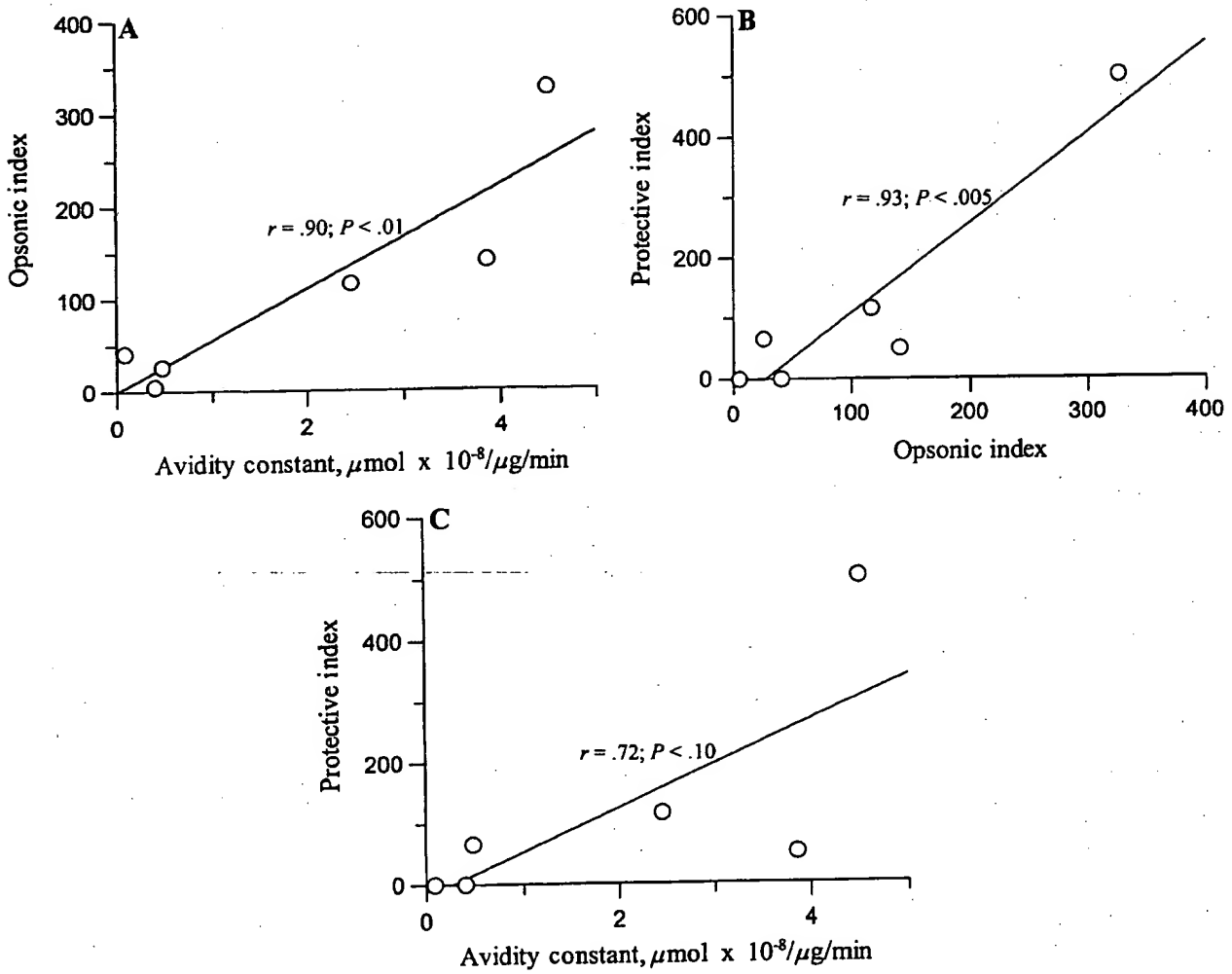

Figure 6. *A*, Relation between avidity constant of IgG and opsonic index (defined in Methods) of sera studied for *Streptococcus pneumoniae* type 3. *B*, Relation between opsonic and protective indices of sera studied for the same serotype. *C*, Relation between avidity constant of IgG and protective index of sera studied for the same serotype.

between avidity and opsonizing activity and between opsonizing activity and mouse protection. If serum from sick or elderly adults had been used in any of these studies, greater discrepancies might have been noted [26]. Of interest, IgG from patients with severe underlying disease was not as likely as that from normal subjects to opsonize pneumococci. It should now be clear why a protective level of IgG has not been determined and why antibody level can not serve as a surrogate for immunity: the degree of immunity may vary greatly, depending upon the functional activity of the antibody, and the population most in need of vaccine may generate antibody that is measured by ELISA but is poorly functional. Our findings underscore the importance of using functional assays, rather than simply measuring IgG levels by ELISA, for studying and comparing polysaccharide vaccines.

On the basis of the pilot study of Yee et al. [32], we studied patients with pneumococcal pneumonia, showing that those with bacteremic pneumonia have an increased risk of homozygosity for the R131 allele of the FCγRIIA surface receptor of PMNL [33]. R131 interacts poorly with Fc of IgG_2, the IgG subclass that includes nearly all IgG to CPS, in contrast to the H131 allelic form, which avidly binds Fc of IgG_2 [34]. Rodriguez et al. [35] found that cultured phagocytic cells transfected with the gene product that encodes H131 ingest opsonized pneumococci more readily than those transfected with the gene for R131. Accordingly, we sought, in the patients included in the present study, to determine whether there was an association between homozygosity for R131 and the presence of IgG to the infecting capsular serotype. Patients who had antibody to the infecting serotype were no more likely to be homozygous

for the R131 allele than were patients who had no antibody. Only 2 bacteremic patients had functional antibody to the infecting serotype at admission, and the FCγRIIA genotype was determined in only 1 of them; this patient was homozygous for R131. Sanders et al. [36] showed that children with the R131 allele may also have reduced IgG_2 responses to pneumococcal vaccine, a finding of further interest in light of our previous and present studies.

Ten patients in this series had received 23-valent pneumococcal vaccine; IgG was detected in acute serum from only 1 of these patients. The absence of antibody in the remaining 9 patients might be consistent with genetic incapacity to respond to a polysaccharide [37], poor response due to age or underlying disease [4, 5], or rapid loss of antibody after an initial response, as suggested by Shapiro et al. [25]. Some researchers [2, 38] have proposed that pneumococcal vaccination protects against bacteremia but not against pneumonia. This concept does not seem to be supported by an understanding of the pathophysiology of the disease because, with inflammation, plasma pours into alveoli, providing a milieu that is not so different from the blood stream. By demonstrating similar rates of antibody presence and lack of function in bacteremic and nonbacteremic subjects, our study further opposes that hypothesis.

We investigated the possibility that anticapsular IgG is not detected in most sera because it is bound in CIC. Such complexes were identified in only 2 of 34 sera examined. Large amounts of CPS can be found in the serum of patients who are hospitalized with pneumococcal pneumonia, and the levels remain high for some time [39]; nevertheless, in most cases, anticapsular IgG also becomes detectable by 5–8 days after the onset of pneumonia and persists for months to years. Although patients with bacteremic or nonbacteremic pneumococcal pneumonia have been found to have elevated levels of CIC [39–41], it has not been shown that these complexes contain IgG to CPS of the infecting serotype. Our study did not exclude the possibility that IgG is bound to capsular material in tissues and does not spill into the circulation.

Acknowledgment

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ERRATA

In an article in the June 2000 issue of the *Journal* (Nzila AM, Nduati E, Mberu EK, et al. Molecular evidence of greater selective pressure for drug resistance exerted by the long-acting antifolate pyrimethamine/sulfadoxine compared with the shorter-acting chlorproguanil/dapsone on Kenyan *Plasmodium falciparum*. J Infect Dis 2000;181:2023–8), the data presented in table 2 are incorrect. The corrected table 2 is shown below.

Table 2. Proportion of dihydropteroate (DHPS) genotypes (expressed as sulfa sensitive and sulfa resistant) of *Plasmodium falciparum* before and after chlorproguanil/dapsone (CPG/DDS) and pyrimethamine/sulfadoxine (PM/SD) treatment.

	% *(n)* DHPS genotypes	
Treatment *(n)*	Sulfa sensitive	Sulfa resistant
CPG/DDS		
Before (28)	82 (23)	18 (5)
After (28)	75 (21)	25 (7)
PM/SD		
Before (24)	79 (19)	21 (5)
After (24)	38 (9)	63 (15)

NOTE. Parasites were collected within 42 days after each treatment, and 24 and 28 pairs of PM/SD and CPG/DDS were analyzed, respectively.

In an article in the July 2000 issue of the *Journal* (Musher DM, Phan HM, Watson DA, Baughn RE. Antibody to capsular polysaccharide of *Streptococcus pneumoniae* at the time of hospital admission for pneumococcal pneumonia. J Infect Dis 2000;182:158–67), the notion that anticapsular antibody may protect against pneumococcal bacteremia without necessarily protecting against pneumococcal pneumonia was incorrectly attributed to reference 2 (Austrian R. Some observations on the pneumococcus and on the current status of pneumococcal disease and its prevention. Rev Infect Dis 1981; 3[Suppl]: S1–17). The authors regret the error.